(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018659 A1

(51) International Patent Classification[7]: C12N 5/08, A61K 39/00, A61P 35/00

(21) International Application Number: PCT/EP2003/009140

(22) International Filing Date: 18 August 2003 (18.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02018512.0 16 August 2002 (16.08.2002) EP

(71) Applicant *(for all designated States except US)*: **GLYCOTOPE GMBH** [DE/DE]; Robert-Rössle-Str. 10, 13125 Berlin (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GOLETZ, Steffen** [DE/DE]; Eichhornstrasse 24, 16548 Glienicke (DE). **BAUMEISTER, Hans** [DE/DE]; Hochsitzweg 159, 14169 Berlin (DE). **SCHÖBER, Ute** [DE/DE]; Kreuzstr. 7, 13187 Berlin (DE).

(74) Agent: **VOSSIUS & PARTNER**; Siebertstrasse 4, 81675 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/018659 A1

(54) Title: PROCESS FOR THE PRODUCTION OF TEMPERATURE-INDUCED TUMOR CELL LYSATES FOR USE AS IMMUNOGENIC COMPOUNDS

(57) Abstract: The present invention relates to a process for the production of an immunogenic compound comprising inducing necrosis by temperature in tumor cells and lysing said necrotic tumor cells so as to obtain a lysate. Furthermore, the invention provides a method for the production of a pharmaceutical composition. Additionally, the invention relates to a pharmaceutical composition comprising a lysate obtainable by the aforementioned process. Moreover, methods and uses for vaccination against cancers, tumorous diseases, infections and/or autoimmune diseases comprising administering the cell lysates of the invention or dendritic cells loaded with the cell lysate loaded to an individual are provided.

Process for the production of temperature-induced tumor cell lysates for use as immunogenic compounds

The present invention relates to a process for the production of an immunogenic compound comprising inducing necrosis by temperature in tumor cells and lysing said necrotic tumor cells so as to obtain a lysate. Furthermore, the invention provides a method for the production of a pharmaceutical composition. Additionally, the invention relates to a pharmaceutical composition comprising a lysate obtainable by the aforementioned process. Moreover, methods and uses for vaccination against cancers, tumorous diseases, infections and/or autoimmune diseases comprising administering the cell lysates of the invention or dendritic cells loaded with the cell lysate loaded to an individual are provided.

Vaccination involves the administration of an agent to an individual, which will stimulate the immune system to react against the "foreign" components of the vaccine. The vaccine can be administered, inter alia, into the skin, muscle, intraorally, subcutaneous, intradermally, intranodally, intraperitoneally, intra- or peritumorally or intravenously. The foreign components of the vaccine are known as "antigens". As a result of a vaccination procedure an individual develops immunity so that a subsequent exposure to the antigen(s) will evoke a response to eliminate or destroy the antigen carrying cells, organisms or particles or improve the disease symptoms or protect from a connected disease. Vaccines have been highly effective in protecting people from infectious organisms. Vaccinations for bacterial and viral infectious agents are now routinely used for: influenza viruses, measles, chicken pox, polio, pneumococcal bacteria, and hepatitis viruses and the like.
Because of the success in immunizing individuals against certain infectious organisms, it has been a great task of clinicians and scientists to develop effective vaccines against cancers.

The fight against infectious diseases with vaccines also teaches that prevention of infectious diseases with vaccines is easier than therapy of the same disease under development. This experience has been interpreted as suggesting that prophylactic vaccination against cancer may be more successful than vaccination when the disease is at an advanced stage. Therefore, several different types of cancer vaccines or immune therapies are under development and are aimed to be used for preventing, ameliorating and/or treating cancer and/or tumorous diseases.
In principle, the assumption of the foregoing was the following: tumor cells can be weakened, or attenuated, and injected like a vaccine into a mammal, e.g. a mouse. Afterwards, if these same tumor cells, at full strength, are injected into the mouse, the mouse will reject or fight the tumor cells and cancer will not develop or decrease the tumor bourdon. However, if a mouse has not been vaccinated, it will develop cancer.

Immuntherapies for preventing, ameliorating and/or treating cancer and tumorous diseases by means of using whole cell vaccines (WCV) have the advantage of being multivalent with respect to tumor-antigens, however WCVs are often only weakly immunogenic. In general, it is to be distinguished between vaccines comprising vital whole tumor cells (WCV) and vaccines comprising lysed tumor cells (TCLV) (Sivanandham (2000), Biological Therapy of cancer, Ed. Rosenberg, S.A., 632-647).

Whole cell vaccines (WCV) have the advantage that tumor cells can be genetically engineered before being vaccinated and, therefore, used as a vehicle for substances that have an immunstimulatory effect (Mach (2000), Curr. Opin. Immunol. 12, 571-575). However, the disadvantages of this therapy are the high technical expense of genetically engineering said cells, the problems of keeping a high quality standard in the production of whole cell vaccines and the ethical problems accompanying the administration of vital tumor cells as vaccines (Sivanandham (2000), loc. cit.).
With respect to WCV, several publications showed that the kind of cell death influences the immune response (Melcher (1999), J. Mol. Med. 77, 824-833). Additionally, it was found that the expression of heat shock proteins influences the

immune response against cells (Galucci (2001), Curr. Opin. Immunol. 13, 114-119; Todryk (2000), Immunology 163, 1398-1408). Moreover, a large number of methods to induce cell-death, in particular, apoptosis, exists which strongly influence the immunogenicity of apoptotic tumor cells (Restifo (2000), Curr. Opin. Immunol. 12, 597-603). With respect to WCV various publications showed that vital necrotic tumor cells are both in vitro and in vivo, i.e. in the animal model system, more immunogenic than apoptotic or untreated cells. In those publications cell death was chemically induced by gancyclovir in tumor cells, which express the herpes simplex virus (HSV) thymidine kinase (HSVtk). Said HSV thymidine kinase functions as a so-called "suicide-gene" if for example induced by gancyclovir. However, cell death only occurs after applying gancyclovir if the cells carrying the recombinant HSV thymidine kinase gene, additionally harbour the anti-apoptotic gene bcl-2. Whereas HSV thymidine kinase positive and bcl-2 negative cells undergo programmed cell death after induction of HSV thymidine kinase by gancyclovir. Only the use of HSVtk and bcl-2 positive whole tumor cells for prophylactic vaccination of mice and the subsequent administration of gancyclovir resulted in a protection of the animals against the development of tumors (Gough (2001), Cancer Res. 61, 7240-7247; Melcher (1998), Nat. Med. 4, 581-587). It was also observed that macrophages phagocytosed both necrotic and apoptotic tumor cells, however, reacted differently with respect to said tumor cells. On one hand immunstimulatory cytokines, like TNFα or IL-1β were secreted if the macrophages phagocytosed necrotic cells, on the other hand immune-suppressive cytokines, like IL-10 were secreted if apoptotic cells were phagocytosed (Gough (2001), loc. cit.). Moreover, macrophages which were co-cultivated either with tumor cells incubated for 1 hour at 45°C or tumor cells incubated for 15 minutes at 45°C were tested for their cytokine secretion pattern. If challenged with tumor cells, which were heat-induced for 1 hour, the macrophages reacted similarly like those challenged with chemically induced necrotic cells with respect to the secretion of cytokines. Whereas tumor cells which were heat-induced for 15 minutes resembled apoptotic cells in that they caused secretion of immune-suppressive cytokines after being phagocytosed by macrophages (Gough (2001), loc. cit.).

In contrast to the above-mentioned findings, it was found that living apoptotic tumor cells are as immunogenic as necrotic tumor cells or even more immunogenic

(Kotera (2001), Cancer Res. 61, 8105-8109; Restifo (2000), loc. cit.; Shaif-Muthana (2000), Cancer Res. 60, 6441-6447); Albert (1998), Nature 392, 86-89). In these publications other methods than those used in the above-mentioned publication for induction of cell death were performed. For example, Kotera (2001) (loc. cit.) could not find a difference between allegedly necrotic cells, which were generated by freezing and thawing and apoptotic cells, which were generated by UV-B treatment when analysing the activation of dendritic cells. The same was observed in an animal model system when investigating the prophylactic and therapeutic efficacy of dendritic cells, which had taken up either the allegedly necrotic, or apoptotic cells. Shaif-Muthana (2000) (loc. cit.) compared the immunogenicity of radioactive irradiated apoptotic cells with allegedly necrotic cells incubated for 30 minutes at 50°C. It was shown that only apoptotic cells could activate T-cells after dendritic cells had phagocytosed them. Restifo (2000) (loc. cit.) pointed out that it depends on the method for induction of apoptosis to generate immunogenic apoptotic cells. In particular, apoptotic cells caused by viral infection showed up to be highly immunogenic.

In summary, in view of the above discussed it appears as if different parameters of whole cell vaccines influence immunogenicity. In particular, whole cell vaccines may arise from apoptotic or necrotic cells and, thus, may have either stimulatory or suppressive effects on the cells of the immune system. Additionally, the efficacy of whole cell vaccines on components of the immune system, in particular, macrophages, dendritic cells or T-cells also varies. Another variable parameter, which seems to influence the immunogenicity of whole cell vaccines, is the kind of technique to induce cell death.

Tumor cells have also been treated with dinitrophenol or fixed with glutaraldehyde in order to improve the immunogenicity before being used as whole cell vaccines (Berd (1997), J. Clin. Oncol. 15, 2359-2370; Sensi (1997), Clin. Invest. 99, 710-717; Fujiwara (1984), J. Immunol. 133, 509-514; Price (1979), Br. J. Cancer 40, 663-665). Additionally, tumor cells have also been treated by physical means, i.e. applying them to high pressure before administration as whole cell vaccines (US 4,931,275). It is of note that these strategies for improving immunogenicity may be problematic in that the used chemicals have to be removed without leaving any residues.

A different approach for treating cancer is hyperthermia. Thereby, tumor tissue is treated with heat. This heat-treatment by is based on the finding that tumor cells are more sensitive to heat than normal cells (Cavaliere (1967), Cancer 20, 1351-1381; Dickson (1979), Lancet 1, 202-205). The aim of many workers in the field of hyperthermia was to demonstrate that tumor cells could be killed in vivo and in vitro by heat. Thus, vital tumor cells were treated with temperatures ranging from 39°C to 46°C to analyse their vitality after the treatment either in cell culture or in an animal model system (Cavaliere (1967), loc. cit.; Giovanella (1970), Cancer Res. 6, 1623-1631; Selawry (1957), Cancer Res. 17, 785-791). Besides the vitality of treated tumor cells, also the immunogenicity of said cells was analysed (Bourdon (1981), Ann. Immunol. 1, 43-63; Mise (1990), Cancer Res. 50, 6199-6202; Check (1974), Cancer 34, 197-203; Mondovi (1972), Cancer, 4 885-888; Price (1979), loc. cit.). Mise (1990) (loc. cit.) has isolated cytotoxic T-cells from mice to which tumor cells have been administered and has analysed the ability of T-cells to lyse tumor cells in vitro. In these experiments it was observed that tumor cells, which had been incubated at 42°C for 30 minutes, were more efficiently lysed than untreated tumor cells. Other studies could also show the beneficial effect of hyperthermia on the lysis of tumor cells by cells of the immune system.

Clinically hyperthermia is used by applying an increased temperature to the tumor in vivo in order to achieve a killing of the cells in vivo. Therefore, during a hyperthermia treatment a patient is not immunized with tumor cell derived vaccines.

Another approach for prophylactic and therapeutic vaccination against tumors are tumor cell lysate vaccines (TCLV). The advantages of tumor cell lysate vaccines over the aforementioned approaches for vaccination and treatment of cancer are the simple method of production, which is not or much less subjected to fluctuations in keeping a high quality standard since tumor cells are killed or lysed before administration. Said killing or lysing can be done mechanically or by freezing/thawing. Additionally, bacterial or viral adjuvants, e.g. Calmette-Guerin (BCG) (Mitchell (1988), Cancer Res. 48, 5883-93) or vaccinia virus (Berthier-Vergnes (1994), Cancer Res. 54, 2433-2439) have been described to improve immunogenicity of tumor cell lysate vaccines.

TCLV are produced by mechanically or enzymatically gaining tumor cells from tumor tissue originating from primary tumor material, metastases and the like to small

pieces and by subsequently lysing and killing them by repeated freezing and thawing - in contrast to whole cell vaccines (WCV). The lysates produced in that manner are so-called "necrotic lysates" (Gallucci (1999), Nat. Med., 11, 1249-55; Kotera (2001), loc. cit.; Restifo (2000), loc. cit.; Sauter (2000), J. Ex. Med., 191, 423-34).

During the process of the "real" necrotic death a series of genes are activated which are only fragmentally known, however, resulting in the secretion of proteins into the medium which show a pro-inflammatory response. However, altered activation of gene expression is highly unlikely to take place during the rapid freeze/thawing as described in the prior art. Therefore, the above-mentioned positive effects assumed to be connected with "real" necrotic cells (Melcher (1999) J. Mol. Med 77, 824-833) and do not apply to the allegedly "necrotic" cell lysates. Thus, the allegedly "necrotic" cell lysates of the prior art do in fact not comprise relevant necrotic cells in the sense of the invention.

Heat shock proteins are amongst the gene products, which are suggested to be involved in the immunological effects of necrotic cells on the immune system (Gallucci (2001), Curr. Opin. Immunol. 13, 114-119; Todryk (2000), loc. cit.). By now many signals have been identified which cause an increased expression of HSPs.

Heat shock proteins (HSPs), belong to the group of stress proteins which are present in all cells in all life forms. They are induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation.

HSPs are also present in cells under perfectly normal conditions. They act like "chaperones," making sure that the cell's proteins are in the right shape and in the right place at the right time. For example, HSPs help new or distorted proteins fold into shape, which is essential for their function. They also shuttle proteins from one compartment to another inside the cell, and transport old proteins to "garbage disposals" inside the cell.

For decades it has been known that animals, e.g. mice can be "vaccinated" against cancer and after many experiments, it was found that one element responsible for immunological responses in mice were heat shock proteins. In particular Hsp70 expression has been shown to be linked with induction of cell death. Thus, Hsp70 seems to be at least one important factor involved in the immunstimulatory effects of necrotic cells (Dressel (2000), J. Immunol. 164, 2362-2371; Melcher (1998), loc. cit.;

Todryk (2000), loc. cit.). Feng (Feng (2001), Blood 11, 3505-3512) has induced the expression of membrane-bound Hsp70 in cells, which caused improvement of the immunogenicity in an animal model system. In a control experiment these cells have been injected as a lysate into mice without provoking an anti-tumor response.
Furthermore, the immunstimulatory role of certain isolated HSPs, in particular Hsp70 and gp96, has been demonstrated (US 6,168,793; US 5,948,646; US 5, 961,979; Schild (2000), Nat. Immunol. 1, 100-101; Binder (2000), J. Immunol. 165, 6029-6035; Wells (2000), Immunol. Today 21, 129-132). In addition, HSPs purified from cell lysates have also been described as highly immunogenic molecules, which present peptides to the immune system. Therefore, they are used commercially for the development of tumor vaccines.

In conclusion, there are problems or drawbacks associated with whole cell vaccines (WCV) or hyperthermia and with tumor cell lysate vaccines (TCLV) and in order to develop or to obtain potent vaccines it is important to increase the immunogenicity dramatically. The medical need and commercial interest for an according efficient tumor vaccine, which can be produced easily, cost efficiently and in a highly reproducible way is therefore given.
Thus, the technical problem underlying the present invention is to provide means and methods for improved vaccination against cancers, tumorous diseases, infections and/or autoimmune diseases.

The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a process for the production of an immunogenic compound comprising the steps of

(a) inducing necrosis by temperature in tumor cells; and

(b) lysing said necrotic tumor cells so as to obtain a lysate.

Surprisingly it was found that induction of necrosis by temperature leads to an increased immunogenicity of tumor cell lysates both in vitro and in vivo. Even more surprising was the finding that tumor cell lysates harbouring a large amount of heat shock proteins are not as immunogenic as tumor cell lysates which were generated

with the inventive process harbouring an amount of heat shock proteins comparable to that of tumor cell lysates from untreated, i.e. non-necrotic tumor cells. This indicates that the expression of heat shock proteins is not correlated with the increased immunogenic effect of the lysates according to the invention.

According to the present invention the term "tumor cells" means cell lines or cells which can be grown under in vitro culture conditions, or tumor cell lines and primary cell cultures, or tumor cells derived from primary or secondary tumor or metastases as listed herein. The cells can be of autologous, allogeneic, syngenic, or xenogenic origin in relation to the person, patient or animal treated and from the same or from different tissues, organs or cell origin in a species (e.g. in case of cancer treatment or prevention in relation to the treated or to be prevented cancer type). The tumor cells used in the process can also be mixtures of the above-mentioned tumor cells. In a preferred embodiment those tumor cells can be altered via mutagenesis, infection with pathogenic particles, like viruses, bacteria, fungi, parasites, or via gentechnological methods, thereby introducing novel antigens or immunogens or parts thereof. In particular, the introduced antigens or immunogens or parts thereof can be of tumors or infectious diseases origin or can be connected with these diseases.
In accordance with the present invention, the term „necrotic tumor cells" means a cell population containing at least 15% necrotic cells as determined for example by the techniques mentioned hereinbelow.

When the tumor cells are derived from tumors or metastases, also including micrometastases, they can, e.g., be obtained by surgery, biopsy, or the like.
The tumor cells can be derived from any possible type of tumors. Examples are skin, breast, brain, cervical carcinomas, testicular carcinomas, head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer etc. The tumor cells may, e.g., be

derived from: head and neck, comprising tumors of the nasal cavity, paranasal sinuses, nasopharynx, oral cavity, oropharynx, larynx, hypopharynx, salivary glands and paragangliomas, a cancer of the lung, comprising non-small cell lung cancer, small cell lung cancer, a cancer of the mediastinum, a cancer of the gastrointestinal tract, comprising cancer of the oesophagus, stomach, pancreas, liver, biliary tree, small intestine, colon, rectum and anal region, a cancer of the genitourinary system, comprising cancer of the kidney, urethra, bladder, prostate, urethra, penis and testis, a gynaecologic cancer, comprising cancer of the cervix, vagina, vulva, uterine body, gestational trophoblastic diseases, ovarian, fallopian tube, peritoneal, a cancer of the breast, a cancer of the endocrine system, comprising a tumor of the thyroid, parathyroid, adrenal cortex, pancreatic endocrine tumors, carcinoid tumor and carcinoid syndrome, multiple endocrine neoplasias, a sarcoma of the soft tissue and bone, a mesothelioma, a cancer of the skin, a melanoma, comprising cutaneous melanomas and intraocular melanomas, a neoplasm of the central nervous system, a cancer of the childhood, comprising retinoblastoma, Wilm's tumor, neurofibromatoses, neuroblastoma, Ewing's sarcoma family of tumors, rhabdomyosarcoma, a lymphoma, comprising non-Hodgkin's lymphomas, cutaneous T-cell lymphomas, primary central nervous system lymphoma, and Hodgkin's disease, a leukaemia, comprising acute leukemias, chronic myelogenous and lymphocytic leukemias, plasma cell neoplasms and myelodysplastic syndromes, a paraneoplastic syndrome, a cancer of unknown primary site, a peritoneal carcinomastosis, a immunosuppression-related malignancy, comprising AIDS-related malignancies, comprising Kaposi's sarcoma, AIDS-associated lymphomas, AIDS-associated primary central nervous system lymphoma, AIDS-associated Hodgkin's disease and AIDS-associated anogenital cancers, and transplantation-related malignancies, a metastatic cancer to the liver, metastatic cancer to the bone, malignant pleural and pericardial effusions and malignant ascites. It is mostly preferred that said cancer or tumorous disease is cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-

related malignancy and/or metastatic cancer. Accordingly, the term "tumor cell" as provided herein, includes in particular a cell afflicted by any one of the above-identified conditions, but is not limited to the mentioned conditions.

The tumor cells are provided in step (a) of the process according to the invention in a form which allows to induce necrosis by temperature.

The tumor cells may be in any state or form, which allows inducing necrosis. They may, e.g., be in the form of fresh material or can be taken from previously frozen material. In a preferred embodiment the previously frozen material in form of cell lines is thoroughly thawed and cultivated. If tumor material is used, it is preferably material which has already been treated mechanically and/or enzymatically so as to provide smaller tissue pieces and/or separate cells.
The resulting cells or tissue pieces are treated afterwards by temperature in order to induce necrosis or are cultivated for a suitable time in vitro.

In the context of the present invention the term "necrosis" means morphological changes of cells. Necrosis is, inter alia, characterized for example by "leakiness" of the cell membrane, i.e. an increased permeability which also leads to an efflux of the cell's contents and an influx of substances perturbing homeostasis and ion-equilibrium of the cell, DNA fragmentation and, finally, to the generation of granular structures originating from collapsed cells, i.e. cellular debris. Typically, necrosis results in the secretion of proteins into the surrounding which, when occurring in vivo, leads to a pro-inflammatory response.

Methods for the determination whether a cell is necrotic or not are known in the prior art and are also described in the examples herein. It is not important which method the person skilled in the art chooses since various methods are known.
However, it is important to distinguish between an apoptotic cell undergoing the so-called programmed cell death and a necrotic cell. Necrotic cells in accordance with the present invention can be determined, e.g., by light-, fluorescence or electron microscopy techniques, using, e.g., the classical staining with trypan blue, whereby the necrotic cells take up the dye and, thus, are stained blue, or distinguish necrotic

cells via morphological changes including loss of membrane integrity, disintegration of organelles and/or flocculation of chromatin. Other methods include flow cytometry, e.g., by staining necrotic cells with propidium iodide. The preferred propidium iodide staining is described in detail in the examples herein. Apoptotic cells can be determined, e.g., via flow-cytometric methods, e.g., attaining with Annexin V-FITC, with the fluourchrome: Flura-red, Quin-2, with 7-amino-actinomycin D (7-AAD), decrease of the accumulation of Rhodamine 123, detection of DNA-fragmentation by endonucleases: TUNEL-method (terminal deoxynucleotidyl transferase caused X-UTP nick labelling), via light microscopy by staining with Hoechst 33258 dye, via Western blot analysis, e.g., by detecting caspase 3 activity by labelling the 89 kDa product with a specific antibody or by detecting the efflux of cytochrome C by labelling with a specific antibody, or via agarose gel DNA-analysis detecting the characteristic DNA-fragmentation by a specific DNA-ladder.
The preferred technologies are described in detail in the examples herein, and are the propidium iodide staining for necrotic cells and the annexin staining for apoptotic cells, whereby apoptotic cells are only stained by annexin V and necrotic cells are stained by propidium iodide and annexin V (Vermes (1995), J. Immun. Meth. 184, 39-51). This flow-cytometry allows rapid and easy qualitative and quantitative measurements.
Preferably, necrosis is determined by flow cytometry, even more preferred is flow cytometry using Annexin V/propidium iodid staining and most preferred is the method as described in the examples herein.

The term "inducing necrosis by temperature" in the context of the present invention means that in a percentage of at least 15%, preferably in more than 40% and herein preferred at least in 70% of the cells of the cell population of the tumor cells changes which result in necrosis.

Methods for measuring said temperature, which is used for inducing necrosis, are known to the person skilled in the art. Preferably, said temperature can be measured by physical means. Said physical means is preferably an optical, mechanical or electrical thermometer, whereby it is understood that normal physical fluctuation in the measurement of a temperature due to errors in the measurement

or physical inertia of the means are in the acceptable range of measurement tolerances. Most preferably, said physical means for measuring temperatures are incorporated by the supplier in the means or in the apparatus used for heat-induction. However, it is also preferred that the temperature may be measured directly within the sample of tumor cells undergoing temperature-induction.

The temperature for inducing necrosis in the cells can be applied to the cells by means and methods known to the person skilled in the art. Possible methods are, e.g., the incubation in heated air or water or irradiation. Suitable means are, e.g., heating blocks, thermal heaters, water bathes incubators, heating rods and the like.

It was surprisingly found that cell lysates produced from tumor cells in which necrosis was induced by temperature as described herein are superior to those of the prior art since their immunogenicity is increased significantly.

It is important to note that the necrotic cell lysates consist of cell populations with the mentioned percentages of relevant necrotic cells as defined above, while cell lysates, which are sometimes called necrotic cell lysates in the literature and which correspond to cells lysed directly without induction of necrosis via rapid freeze/thawing, are called non-treated cells or non-treated cell lysates in the examples herein.

In accordance with the present invention the term "lysing" relates to various methods known in the art for opening/destroying cells. The method for lysing a cell is not important and any method that can achieve lysis of the tumor cells may be employed. An appropriate one can be chosen by the person skilled in the art, e.g. opening/destruction of cells can be done enzymatically, chemically or physically. Non-limiting examples for enzymes and enzyme cocktails are proteases, like proteinase K, lipases or glycosidases; non-limiting examples for chemicals are ionophores, like nigromycin, detergents, like sodium dodecyl sulfate, acids or bases; and non-limiting examples of physical means are high pressure, like French-pressing, osmolarity, temperature, like heat or cold. Additionally, a method

employing an appropriate combination of an enzyme other than the proteolytic enzyme, an acid, a base and the like may also be utilized.

According to the present invention the term "lysate" means a solution or suspension in an aqueous medium of cells that are broken. However, the term should not be construed in any limiting way. The cell lysate comprises, e.g., macromolecules, like DNA, RNA, proteins, peptides, carbohydrates, lipids and the like and/or micromolecules, like amino acids, sugars, lipid acids and the like, or fractions of it. Additionally, said lysate comprises cell debris which may be of smooth or granular structure. The details of the preparations are specifically described in the examples of the present specification. Accordingly, those skilled in the art can prepare the desired lysates by referring to the above general explanations and specific explanations in the examples, and appropriately modifying or altering those methods, if necessary. Preferably, said aqueous medium is water, physiological saline, or a buffer solution that any solid mass cannot be observed without help of optical means, and that the dispersoids can be phagocytosed by the antigen-presenting cells. An advantage of the tumor cell lysate obtainable by the processes of the present invention is that it can be easily produced as described in the appended Examples and stored cost efficiently since less technical facilities are needed.

However, said lysate is not limited to necrotic cells since, for example, due to the different sensitivity of the treated cells or due to the applied conditions for the temperature-induction of cells also apoptotic cells can be part of the cell population from which the lysate is obtained. Nevertheless, necrotic cells have to be at least 15% of the cell population, preferably more than 40%, particularly more than 70%. The determination of the percentages may vary from experiment to experiment due to measuring inaccuracies occurring in the technology of flow cytometry and the possibility of varying interpretations of the data and setting of the gates. Accordingly, a deviation of 10%, preferably of 5% and more, preferably of 1% may occur if a method other than that specifically described in the examples is used.

Preferably, the tumor cells are lysed by freezing and thawing, more preferably freezing at temperatures below -70°C and thawing at temperatures of more than 30°C, particularly freezing is preferred at temperatures below -75°C and thawing is preferred at temperatures of more than 35°C and most preferred are temperatures for freezing below -80°C and temperatures for thawing of more than 37°C. It is also preferred that said freezing/thawing is repeated for at least 1 time, more preferably for at least 2 times, even more preferred for at least 3 times, particularly preferred for at least 4 times and most preferred for at least 5 times.

According to the invention, lysates are also preparations of fractions of molecules from the above-mentioned lysates. These fractions can be obtained by methods known to those skilled in the art, e.g., chromatography, including, e.g., affinity chromatography, ion-exchange chromatography, size-exclusion chromatography, reversed phase-chromatography, and chromatography with other chromatographic material in column or batch methods, other fractionation methods, e.g., filtration methods, e.g., ultrafiltration, dialysis, dialysis and concentration with size-exclusion in centrifugation, centrifugation in density-gradients or step matrices, precipitation, e.g., affinity precipitations, salting-in or salting-out (ammoniumsulfate-precipitation), alcoholic precipitations or other proteinchemical, molecular biological, biochemical, immunological, chemical or physical methods to separate above components of the lysates. In a preferred embodiment those fractions which are more immunogenic than others are preferred. Those skilled in the art are able to choose a suitable method and determine its immunogenic potential by referring to the above general explanations and specific explanations in the examples herein, and appropriately modifying or altering those methods, if necessary.

The induction of necrosis by temperature according to step (a) of the method according to the invention is preferably achieved by incubation of the tumor cells at a temperature which is above the average body temperature of the organism from which the cells are derived, preferably at a temperature above 37°C. More preferably, the cells are incubated at a temperature of more than 38°C, even more preferably of more than 39°C, particularly preferred of more than 40°C and most preferred of more than 41,5°C.

It is preferred that the temperature is chosen in a way that although necrosis is induced, no or only a low increase in hsp70 expression is induced. By a "low increase" an increase of preferably not more than a factor of 2 is meant. The corresponding temperature can depend on the specific cell type used but can be easily determined by the skilled person by routine experimentation, e.g. by incubating the corresponding cells at different temperatures and determining the amount of necrotic cells and the level of hsp70 expression at different temperatures using methods as those shown in the examples herein.

In an even more preferred embodiment the tumor cells are incubated at a temperature of more than 41.2°C, more preferably at a temperature of more than 42°C, more preferably at a temperature in the range of 45°C to 55°C, even more preferred in the range of 45.5°C to 47°C. In a most preferred embodiment the cells are incubated at a temperature in the range of 46.0°C to 46.4°C, in particular at about 46.2°C.

As shown in the examples herein, temperatures of about 46.2°C used for inducing necrosis lead to a surprisingly high amount of necrotic cells and an unexpectedly high increase in immunogenicity.
Moreover, it is demonstrated in the examples herein that tumor cell lysates produced from tumor cells in which necrosis has been induced by temperature of about 46.2°C have also surprising effects in vivo. Comparable results were also achieved with cell lysates from the same cell line induced at 46°C.
In particular, mice immunized with the tumor cell lysates of the present invention showed immune reactions against the antigens present on the tumor cells used for the production of the cell lysates. Said mice developed antibodies of the IgG-type that can only be produced if a so-called class-switch has occurred. Said class-switch is only possible if T helper cells are included in the immune response to antigens. Moreover, it was also surprisingly found that the immunized mice developed said IgG-type antibodies against carbohydrate antigens for which so far only IgM-type antibodies have been developed by mice. However, IgM-type antibodies can be produced without a class-switch that implies that T helper cells had not been involved. Thus, the tumor cell lysates of the present invention are

advantageous as regards immunogenicity and are favourable for vaccination against cancer, tumorous diseases, infections and/or autoimmune diseases since they evoke an immune response in which also B- and T-cells are involved.

The incubation of the tumor cells at a specific temperature is carried out for a period of time sufficient to induce necrosis and depends on the temperature and the means and methods for producing it. It is of note that the person skilled in the art knows that the time needed for induction of necrosis in said tumor cells may vary depending, inter alia, on the cells used, the status of the cells, the conditions in the culture medium, the sensitivity of the cells and the like. Moreover, the time for induction of necrosis may also depend on the kind of applying the temperature to the cells and on the apparatus used for applying the temperature. Moreover, the time for inducing said necrosis is also dependent on the temperature at which the cells have been incubated for the induction of necrosis. The most preferable temperature may also vary depending on the type and source of the tumor cells.

Generally, the incubation should last for a period of time of at least 1 minute. Preferably, the induction lasts for a period of time of at least n minutes, wherein n is an integer in the range of 2 to 60, with n=15 being particularly preferred. More preferably, the incubation lasts at least 1 hour, even more preferred at least 2 hours and particularly preferred at least 3 hours. More preferably between 1.5 and 5 hours. The most preferred incubation is between 2 and 3 hours.

There is in principle no upper limit for the time of incubation. However, it is preferably no longer than 48, 36, 24, 12, 11, 10, 9, 8, 7, 6, 5 or 4 hours.

In an advantageous preferred embodiment the necrotic cells are directly lysed after the temperature induction of necrosis after temperature-induction of necrosis.

In a preferred embodiment the necrotic cells obtained after step (a) of the process according to the invention are further incubated before lysing them. As shown in the examples herein, this further incubation also influences the amount and immunogenicity of necrotic cells. Said further incubation is preferred to be

performed at a temperature of more than 35°C, even more preferably at a temperature of more than 36°C, particularly preferred at a temperature of about 37°C. The time for this further incubation is preferred to be at least 1 hour, more preferably to be at least 2 hours, even more preferred to be at least 3 hours, particularly preferred to be at least 6 hours, even more particularly preferred to be at least 12 hours and most preferred to be at least 22 hours.

In a preferred embodiment of the invention, it is envisaged that in the process of the present invention more than 15% of said induced tumor cells are necrotic.
As mentioned herein above, due to, e.g., the different sensitivity of the treated cells, due to the applied conditions for the temperature-induction of cells or due to different conditions in culturing the tumor cells that consist of a population of the cells also comprising apoptotic cells which are subsequently lysed together with the other cells of the population also comprising the necrotic cells according to the described process.

In order to obtain an effective immunogenicity it is desirable to obtain a certain percentage of necrotic cells in the lysate. Accordingly, the process is preferably designed so as to result in more than 15, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 82, 84, 86, 87, 88, 89, 90, 92, 94, 96, 98, 99% of the temperature-induced tumor cells being necrotic. Most preferably, more than 40% or 70% of said tumor cells are necrotic. It is of note that the amount of necrotic cells can also depend on the temperature, time and time for regeneration after induction of necrosis by temperature as demonstrated in the examples herein as well as on the type and source of the tumor cells.
Also preferred is that the tumor cells used in the process according to the invention are genetically engineered, mutated or infected by oncogenic viruses.
In the context of the present invention the term "genetically engineered" is used in its broadest sense for methods known to the person skilled in the art to modify desired nucleic acids in vitro and in vivo such that genetic modifications are affected and genes are altered by recombinant DNA technology. Accordingly, it is preferred that said methods comprise cloning, sequencing and transformation of recombinant nucleic acids. For this purpose appropriate vectors, primers, enzymes, host cells

and the like can be used and are known by the skilled artisan. Preferably, genetically engineered tumor cells comprise cells harbouring recombinant nucleic acids encoding antigens or immunogens or parts thereof, cytokines, chemokines, growth factors and the like. Antigens and immunogens can be, for example, one or more tumor antigens or parts thereof, antigens from infectious microorganisms or parasites, like bacteria, fungi, viruses and the like. Furthermore, among the immunogens are, for example, molecules which increase the immunogenicity, like pan T-cell epitopes or multimers thereof, like PADRE-epitopes, or tetanus toxoid fragments which evoke an additional immunstimulatory effect via activation of MHC class II-mediated processes.

Also preferred are tumor cells genetically engineered with nucleic acids encoding effector molecules, like transcription factors, components of signal transduction pathways or signalling cascades, or cytokines, chemokines, growth factors and the like which are able to modulate directly or indirectly the expression of endogenous molecules, e.g. nucleic acids, polypeptides, posttranslationally modified polypeptides and lipids and the like. More preferably, the tumor cells are transiently or stably transfected with a desired nucleic acid molecule.

It is also preferred that the tumor cells are genetically engineered so as to express a polypeptide against which antibodies should be raised. If cell lysates from these tumor cells are produced and administered to an individual, it is expected that a humoral and/or cellular immune response is developed by individuals, preferably this immune response comprises antibody responses and/or T helper cell responses and/or cytotoxic T cell responses.

In accordance with the present invention, the term "mutated" means (a) permanent modification(s) of genetic material, i.e. nucleic acids, caused, for example, naturally or by physical means or chemical compounds/substances/agents. Said modifications include point mutations, like transitions or transversions, deletion/insertion/addition of one or more bases within a nucleic acid/gene/chromosome thereby modifying the nucleic acid/gene/chromosome which can cause, inter alia, aberrant gene expression/transcription/translation or inactive gene products, constitutive active/inactive gene products leading to e.g. dominant-negative effects. Thus, it is also preferred that the tumor cells comprise cells which

harbour (a) mutation(s) in (a) desired gene(s) or in which (a) mutation(s) in (a) desired gene(s) is induced by methods known to the person skilled in the art. It is also known in the prior art that mutated or genetically engineered tumor cells can be selected by any suitable method/phenotype.

In accordance with the present invention the term "infected" means cells, which have been infected with a virus, or viroid, and/or proteinaceous structure. Said virus, or viroid, and/or proteinaceous structure may also be used as a vehicle for genetically engineering said cells. It is preferred that said virus which infects tumor cells is an oncogenic virus, however, is not limited to oncogenic viruses. Most preferably said oncogenic virus is selected from the group consisting of retroviruses or DNA viruses, e.g. papovaviruses like human papilloma viruses (HPV), type C oncoviruses, like human T cell leukaemia viruses (HTLV), herpes viruses, like Epstein-Barr virus (EBV), hepadnaviruses, like hepatitis B virus (HBV), and lentiviruses, like human deficiency virus (HIV). It is also preferred that tumor cells are already infected with any one of the above mentioned viruses. Furthermore, infected tumor cells or lysates thereof may be important when used for prophylactic/therapeutic vaccination against infectious diseases caused for example by viruses like HIV, HBV, hepatitis C virus (HCV), HPV. Preferably, the infectious component(s) comprised by the lysates produced from these infected cells has/have to be additionally inactivated. Methods to be used are known to those skilled in the art, e.g., heat inactivation, acid inactivation and/or sterile filtration or the like.

Moreover, in another preferred embodiment of the present invention the tumor cells are autologous.

In the context of the present invention the term "autologous" means that the tumor cells are derived from the same individual to which the lysate resulting from the process according to the invention shall be later administered. One advantage is by using cells from the autologous tumor that there are a large variety of relevant and suitable antigens in the lysate for treating cancers or tumorous diseases of the individual or prevent its recurrence or relapse.

Alternatively, in another preferred embodiment the tumor cells are allogeneic. In the context of the present invention the term "allogeneic" means that the tumor cells are

derived from an individual which is different from the individual to which the lysate resulting from the process according to the present invention shall be later administered. A herein further preferred embodiment of the allogeneic tumor cells for use in an allogeneic setting are tumor cells of a patient, or cells originating from these cells, which were successfully used for immunization of that latter patient in a autologous setting resulting in a partial or complete reduction of tumor load.

In the context of the present invention the term "allogeneic tumor cells" further comprises cell lines, including cell lines, e.g., tumor cell lines, or cell lines or cultures from primary material and the like, which are not originating from the individual to which the lysate shall be administered. The advantage of allogeneic tumor cells are antigens or immunogens which are not shared by the cancers or tumorous diseases to be treated or prevented, resulting in an immune response comprising a strong danger signal and/or helper response which can be favourable to overcome anergies or tolerances.

Alternatively, in a preferred embodiment the tumor cells are xenogenic. In the context of the present invention the term "xenogenic" means that the tumor cells comprise tumor cells from primary or secondary tumors or from metastases, cell lines, e.g., tumor cell lines, immortalized cell lines or cell lines or cultures from primary material and the like, which are not originating from the same species to which the lysate shall be later administered.

Alternatively, in another preferred embodiment the tumor cells are allogeneic, autologous or xenogenic and are of a different tissue or cell source or the like than the cancers or tumors, e.g. tumor cells from a mammary tumor cell line are used for generating a lysate according to the process of the invention which is administered to an individual for prophylaxis or treatment of colon or gastric cancers or tumorous diseases, or a lysate according to the invention from a mutated myeloma cell line for use in an individual for prophylaxis or treatment of carcinomas. Preferably, these cell lines have one or more antigens which are shared with the cancers or tumorous diseases to be treated. The advantage is an additional strong response against antigens or immunogens foreign to the cancers or tumorous diseases to be treated or prevented, e.g., antigens specific for the tumor cell which are not shared by the cancer or tumors to be treated or prevented, comprising a strong danger signal

and/or helper response which can be favourable to overcome anergies and/or tolerances. In a preferred embodiment the tumor cells are in addition from an allogeneic source which can in addition have a strong allo-response which can be further favourable to overcome anergies and/or tolerances.

In a preferred embodiment, the lysate is prepared from tumor cells of different types or different lysates prepared from different types of tumor cells are used in combination for the administration to an individual.

More preferably the tumor cells of the lysate or the combination of lysates are allogeneic and autologous tumor cells. Even more preferably, they are a mixture from tumor cells from the same tissue or cell source and the like together with those from a different tissue or cell source and the like. It is, e.g., possible to use a cell lysate from an allogeneic mutated myeloma cell line with a lysate from colon cancer cells from the individual to which the lysate(s) shall be later administered. The advantage is the combination of large amounts of shared antigens from autologous material with the allogeneic part and, therefore, an increased helper response, allo-response and/or danger signals in order to break potential tolerances and/or anergies of the cancers or tumors. Another example is the mixture of the above allogeneic myeloma cell line with another colon or gastric cancer cell line for the administration to an individual to treat or prevent colon carcinoma. The advantage of the latter is the possibility to generate a potent "off-the-shelf" vaccine with a mixture of large amounts of tumor antigens and an increased helper response, allo-response and/or danger signals in order to break potential tolerances and/or anergies.
The tumor cells can be mixed before the induction of necrosis by temperature induction or after induction but before the lysis or after the lysis. The skilled artisan is able to determine which is favourable for the usefor the production, regulatory issues, application to an individual and for its cancers or tumorous diseases.

The examples of the present invention demonstrate that both, an autologous and allogeneic system, can be used for increasing the imunogenicity. As described hereinabove, the process of the present invention allows to produce highly efficient

immunogenic compounds by inducing necrosis in the tumor cells and subsequently lysing the cells. The obtained tumor cell lysates can be used for the therapeutic or prophylactic treatment of cancer, tumorous diseases, infections and /or autoimmune diseases.

In this contex the term "immunogenic compound" means compounds having the ability to evoke immune reactions of the cells of the immune system like macrophages, dendritic cells, Langerhans' cells, B- (B1 and B2) and/or T-cells (cytotoxic T cells (Tc), T-helper cells (Th0, Th1 and Th2)), natural killer cells (NK cells), memory cells and the like Preferably, the term "immunogenic compound" means compounds having the ability to evoke humoral and/or cellular immune response, wherein at least one of the cells/group of cells of immune effector cells or cell products of said immune effector cells, for example one or more of the aforementioned cells are involved. The person skilled in the art is aware of various methods to determine whether an immune response is evoked. Examples for methods used for this purpose are shown in the examples and be transferred, where necessary by those skilled in the art, to determine the response in an individual or patient treated with the lysates according to the invention. Furthermore, other methods known to those skilled in the art can complement these techniques.

In general, an immunogenic compound leads to an immune response comprising humoral and/or cellular responses; normally comprising that genes or gene products that affect the level of immune responses are expressed/activated, e.g. those of the major histocompatibility class (MHC) I and II, those of antibody light and heavy chains, those of members of the immunglobulin superfamily, those of T-cell receptor/receptor compounds, those of cytokines or those of signal transduction cascades involved in transmitting immune responses.

In a preferred embodiment, the tumor cells used for the production of a cell lysate as described herein are NM-F9 (DSMZ deposit No.____) or NM-D4 cells (DSMZ deposit No. ____).

The term "NM-F9" (also referred herein as "F9" or "TF-positive F9 cells") or "NM-D4" means cell lines or cells derived from the human myelogenous leukemia cell line K562 (ATCC: CCL-243). NM-F9 and NM-D4 were deposited with the Deutsche

Sammlung für Mikroorganismen und Zellkulturen GmbH ("DSMZ") on _____, 2003. The DSMZ is located at the Mascheroder Weg 1b, D-38124 Braunschweig, Germany. The DSMZ deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

The present invention also relates to a lysate obtainable or obtained by the process according to the present invention and to dendritic cells loaded with such a lysate.

Moreover, the present invention also relates to a composition comprising a lysate or dendritic cells according to the present invention.

In a preferred embodiment said composition is a pharmaceutical composition. In accordance with the present invention the term "pharmaceutical composition " relates to compositions comprising the cell lysates described hereinabove which are obtained by the aforementioned processes and having the desired pharmacological activity. Such pharmaceutical compositions comprise a therapeutically effective amount of the cell lysates of the present invention, and a pharmaceutically acceptable carrier. The pharmaceutical composition may be administered with a physiologically acceptable carrier to a patient, as described herein. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also

contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the cell lysate, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In another preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilised powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The cell lysate of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the cell lysate of the invention which will be effective in the treatment or prevention (in particular by vaccination) of cancers, tumors, tumorous diseases,

infections and/or autoimmune diseases can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

In another preferred embodiment the composition is a vaccine composition.

In accordance with the present invention the term "vaccine composition" relates to any composition which can be used as a vaccine.

The forms or methods for manufacturing vaccine compositions according to the present invention are not particularly limited, and a composition in a desired form can be prepared by applying a single method available in the field of the art or methods in an appropriate combination. For the manufacture of a vaccine composition, aqueous media such as distilled water for injection and physiological saline, as well as one or more kinds of pharmaceutical additives available in the field of the art can be used. For example, buffering agents, pH adjusting agents, solubilizing aids, stabilizing agents, soothing agents, antiseptics and the like can be used, and specific ingredients thereof are well known to those skilled in the art. The vaccine composition can also be prepared as a solid preparation such as a lyophilized preparation, and then prepared as an injection by adding a solubilizing agent such as distilled water for injection before use. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways as discussed below. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt %. The vaccine composition may be administered alone or in combination with other treatments, i.e., radiation, or other chemotherapeutic agents or anti-cancer agents.

In a preferred embodiment, the vaccine compositions are in a water-soluble form, such as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts.

The vaccine compositions can be prepared in various forms, such as injection solutions, tablets, pills, suppositories, capsules, suspensions, and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. The vaccine compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavouring agents; colouring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations.
A vaccine composition according to the present invention can be used for immunization against cancer, tumorous diseases, autoimmune diseases and/or infectious diseases.

In another preferred embodiment the composition according to the present invention, in particular the vaccine composition, is optionally combined with an adjuvant or dendritic cells.
In accordance with the present invention "dendritic cells" relate to professional antigen-presenting cells which capture antigens and migrate to the lymph nodes and spleen, where they are particularly active in presenting the processed antigen to T cells. The term "dendritic cells" also means cells which have an activity and function similar to dendritic cells. Dendritic cells can be derived from either the lymphoid or mononuclear phagocyte lineages. Said dendritic cells can be found in lymphatic and non-lymphatic tissue. The latter appear to induce a T cell response only when being activated and having migrated to lymphatic tissues.

Dendritic cells are known to be the or amongst the most potent activators and regulators of immune responses. One important feature is that they are presently the only antigen presenting cells known to stimulate naïve T cells. Immature dendritic cells are characterized by their ability to take-up and process antigens, a

function that is dramatically reduced in mature dendritic cells, which in turn exhibit enhanced presentation of processed antigens on their surface, mainly bound to MHC Class I and Class II molecules. Maturation is also associated with upregulation of costimulatory molecules (such as CD40, CD80 and CD86), as well as certain other cell surface proteins (e.g. CD83 and DC-Sign). Dendritic cell maturation is also usually associated with enhanced migratory capacity, resulting (in vivo) in migration of dendritic cells to the regional lymph nodes, where the dendritic cells encounter T and B lymphocytes. Dendritic cells can be obtained from individuals using methods known to those skilled in the art and are described in more detail in the examples herein. Furthermore, according to the invention, dendritic cells are also those cells or cell lines which show the comparable functional and/or phenotypic features as dendritic cells, e.g. MUTZ-3 derived cells.

Dendritic cells or their precursors are differentiated using suitable growth factors and/or cytokines, e.g. GM-CSF and IL-4 as shown in the examples herein, the resulting immature dendritic cells are loaded with a lysate according to the invention. Immature DC (iDC) loaded with a lysate according to the invention are further maturated to mature DC (mDC). In special cases also mDC can be loaded (pulsed) with antigens or immunogens from the lysate. Vaccine compositions or pharmaceutical compositions for preventing or treating cancers, tumorous diseases and or infectious diseases preferentially comprise loaded mDC which originate from loaded and matured iDC or which were loaded after or during maturation. Vaccine compositions for autoimmune diseases preferentially comprise loaded iDC which are preferably transiently or stably arrested in the iDC state using methods known to those skilled in the art.

In a preferred embodiment of the invention treatment or prevention of the cancers, tumorous diseases, infections and/or autoimmune diseases and in particular for cancers , tumorous and infectious diseases combines dosages comprising a lysate according to the invention loaded onto dendritic cells with dosages comprising only a lysate according to the invention. The advantage is to combine the ex vivo loading of autologous or allogeneic dendritic cells with the in vivo "loading" of dendritic cells which occurs via the administration of the lysate to an individual. Thereby, different

application routes might be preferable. The administration of dendritic cells directly to lymphnodes or other areas with direct contact to the important immune cells to be stimulated by dendritic cells are preferred. The administration of the lysate intradermally, subcutaneously or intrarectally to Payers patches or other areas where dendritic cells or their precursors are located and preferably concentrated, is preferred.

Another preferred embodiment of the invention are vaccine compositions or pharmaceutical compositions comprising a lysate according to the invention loaded onto suitable dendritic cells and adjuvants or costimulatory factors for the enhancement of the action of the dendritic cells, e.g. GM-CSF, interleukins.

With respect to the present invention the term "adjuvant" means that the natural ability of an antigen to induce an immune response can be modified, and in particular enhanced, by altering or by mixing it or loaded dendritic cells described hereinabove with another substance. The term "adjuvant" also means that tumor cells from which the lysates are generated and/or dendritic cells are genetically modified in order to express adjuvants or other factors which influence the immune response, as for example costimulatory factors. The procedure or the substance used to enhance immune responses is called an adjuvant. At least three classes of adjuvants have been used for a long time; these are mineral oil emulsions, aluminium compounds, and surface active materials such as saponin, lysolecithin, retinal, Quil A.RTM., some liposomes, and pluronic polymer formulations. See, for example, Fundamental Immunology, edited by William E. Paul, at p. 1008, Raven Press, New York (this book will hereinafter be referred to as "Fundamental Immunology"). Aluminium adjuvants used alone or in combination include aluminium hydroxide gel, aluminium phosphate, aluminium sulphate, and alums comprising ammonium alum (such as (NH.sub.4).sub.2 SO.sub.4.Al.sub.2 (SO.sub.4).sub.3) and potassium alum. Aluminium hydroxide (hereinafter "AL") is one of the older adjuvants and it is considered so safe that it has been applied in bacterial and viral vaccines administered to billions of people around the world. Calcium phosphate gel (hereinafter "CP") has similar properties and is also used in vaccines. Both substances are available in pharmaceutical qualities in most countries worldwide. Techniques for preparing adjuvant-antigen preparations for injection are well known

in the art. See, for example, Terry M. Phillips, Analytical Techniques in Immunochemistry, pp. 307-10, Marcel Dekker, New York, 1992. Other adjuvants include complete Freund's adjuvant (a water-in-oil emulsion in which killed, dried, mycobacteria--usually M tuberculosis--are suspended in the oil phase); incomplete Freund's adjuvant (analogous to the complete Freund's adjuvant with no mycobacteria); ISCOM (or immune stimulating complex, comprising lipophilic particles formed by the spontaneous association of cholesterol, phospholipid and the saponin Quil A.RTM.); lipopolysaccharide (complex molecules consisting of a lipid core--lipid A--with a polysaccharide side chain that are components of certain bacilli, Lipid A is incorporated into the outer membrane of the bacterium and the polysaccharide projects extracellularly. Their adjuvant potency is associated with lipid A; they are also mitogenic for murine B lymphocytes); and mycobacterial adjuvants (whole, heat killed, dried, mycobacteria - such as M. tuberculosis, M. avium, M. phlei, and M. smegmatis) that, when suspended in mineral oil and emulsifier, have adjuvant activity with respect to any antigen given with them. Extracts of some mycobacteria, e.g., mycobacterial peptidoglycolipids have similar adjuvant activities. See, for example, Dictionary of Immunology at pp. 3, 7, 46, 94, 97, 105, and 116; R. B. Luftig, Microbiology and Immunology, pp. 228-29, Lippincott-Raven Publishers, Philadelphia 1998. Microbial adjuvants include Corynebacterium parvum and Bordetella pertussis. See, for example, Handbook of Immunology at 115-16. Use of controlled-release preparations and materials with adjuvant activity and possible sites of action have been described in Fundamental Immunology at pp. 1007-09. Mineral carriers such as aluminium hydroxide, potassium ammonium sulphate, and potassium aluminium sulphate adsorb the antigen on their surface. These common adjuvants have been used at a 0.1% concentration with up to 1 mg protein antigen in 1 ml administered to animals at doses of 0.2-0.5 ml/(kg body weight). See Miroslav Ferencik, Handbook of Immunochemistry, p. 115, Chapman & Hall 1993 (this book will hereinafter be referred to as "Handbook of Immunochemistry"). Although Freund's adjuvant is toxic and not used for immunization of human beings, mineral adjuvants such as aluminium hydroxide are common in human medicine. Id. at 116. In addition to alum, other adjuvants in the group of inert carriers include bentonite, latex, and acrylic particles. See Fundamental Immunology at 1008. Combinations of adjuvants

can also have adjuvant properties. For example, it has been shown that the combination of saponin and muramyl dipeptide in a squalene in water emulsion is superior to alum as an adjuvant for inducing certain responses in mice. R. Bomford, M. Stapleton, S. Wilson, A. McKnight, and T. Andronova, The control of the antibody isotype responses to recombinant human immunodeficiency virus gp120 antigen by adjuvants, AIDS Res. Hum. Retroviruses Vol. 8(1992) pp. 1765 et seq. These adjuvants are complemented by new adjuvants that have been developed during the last fifteen years. See, for example, Anthony C. Allison, The Role of cytokines in the Action of Immunological Adjuvants, in Vaccine Design. The Role of cytokine Networks, edited by Gregory Gregoriadis and Brenda McCormack, NATO ASI Series A: Life Sciences Vol 293, pp. 1-9, Plenum Press, New York 1997 (this book will hereinafter be referred to as "Vaccine Design"); Immunology at p. 116; H. Snippe, I. M. Fernandez and C. A. Kraaijeveld, Adjuvant Directed Immune Specificity at the Epitope Level. Implications for Vaccine Development. A Model Study Using Semliki Forest Virus Infection of Mice, in Vaccine Design at pp. 155-73. An adjuvant can be administered prior to, simultaneously with, or following the administration of the antigen. Antibody production enhancement caused by adjuvants is not fully understood. However, adjuvant properties that may exist either alone or in various combinations and which permit a substance or formulation to be described as adjuvant active have been defined. See, for example, J. C. Cox and A. R. Coulter, Adjuvants--A classification and review of their modes of action, Vaccine Vol. 15(1981) pp. 248 et seq.; John Cox, Alan Coulter, Rod Macfarlan, Lorraine Beezum, John Bates, Tuen-Yee Wong and Debbie Drane, Development of an Influenza-ISCOM.TM. Vaccine, in Vaccine Design at pp. 33-49. One of these properties is depot generation, whereby the vaccine is retained near the dose site to give short-term trickle release or a longer term pulsed release. Id. at p. 34.

Preferably, the pharmaceutical or vaccine composition is administered directly or in combination with an adjuvant mentioned herein above or loaded on antigen-presenting cells, particularly dendritic cells. It is also preferred that both the pharmaceutical or vaccine composition and the adjuvant and the pharmaceutical or vaccine composition and the loaded dendritic cells are administered together or separately from each other e.g. at different time points or at different locations.

Additionally, it is also preferred that said pharmaceutical composition and adjuvant is administered together with said pharmaceutical composition loaded on dendritic cells. Since dendritic cells are highly specialized antigen-presenting cells with the unique capability in initiating and regulating antigen-specific immune responses, it is preferred to combine them with the pharmaceutical or vaccine compositions of the present invention. For the preparation of a tumor vaccine dendritic cells can be generated from the peripheral blood of tumor patients from other donors or from the above-mentioned cell lines. In clinical studies, the efficacy of vaccination with dendritic cells has been demonstrated using immunological and - in some cases - clinical endpoints.

Active specific immunotherapy approaches to the treatment of tumors have been widely investigated during recent years. Numerous studies involving the vaccination of patients with their own inactivated tumor cells have been reported. These studies have demonstrated that inclusion of an adjuvant is necessary to stimulate the patient's immune system especially against the autologous, or derived from self, tumor cells. For example, methods utilizing the particulate adjuvant, Bacillus Calmette-Guerin (BCG) cells, administered systemically or mixed with the patient's own tumor cells have been shown to induce tumor-specific immunity in laboratory animals. Peters, L. C., Brandhorst, J. S., Hanna Jr., M. G., Preparation of Immuno-Therapeutic Autologous Tumor Cell Vaccines from Solid Tumors; Cancer Res. 39: 1353-1360 (1979).

In another preferred embodiment the dendritic cells used in the aforementioned pharmaceutical or vaccine composition are loaded mature dendritic cells (mDC) which originate from lysate-loaded and further matured immature dendritic cells (iDC) or which were loaded after or during maturation. The term "immature" when used in accordance with the present application relates to professional antigen-presenting cells that are characterized by their ability to take-up and process antigens. The term "mature" when used in accordance with the present application relates to professional antigen-presenting cells that express costimulatory factors and antigens in the context of MHC class molecules or CD1 molecules and can activate T cells, regulatory NKT cells and/or B cells.]

A pharmaceutical or vaccine composition comprising dendritic cells and temperature-induced cell lysates, comprises them preferably as dendritic cells loaded with lysate. Said dendritic cells are preferably loaded in their immature stadium (immature DC) with the cell lysates of the present invention and are subsequently being brought to maturation. Mature DC loaded with the lysate according to the invention are preferably used to treat or prevent tumorous or infectious diseases. In case, the pharmaceutical or vaccine composition is used for vaccination against autoimmune diseases the immature DC are loaded and preferably arrested in their immature stadium to develop tolerance when being administered as already described hereinabove.

Moreover, the present invention relates to a method for the production of a vaccine composition comprising the step of combining a cell lysate obtainable by the process according to the present invention with an adjuvant or with dendritic cells.

The present invention also relates to a method for the production of a pharmaceutical composition comprising the step of combining a cell lysate obtainable by the process according to the present invention with a pharmaceutically acceptable carrier.

In another aspect the present invention relates to a method for the treatment or prevention, e.g. by vaccination, of cancer, tumorous diseases, infections and/or autoimmune diseases in an individual comprising the step of administering to the individual a therapeutically or prophylactically effective amount of the lysate obtainable by the process according to the invention.

In the context of the present invention the term "individual" means a subject in need of a treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases. Preferably, the subject is a vertebrate, even more preferred a mammal, particularly preferred a human.

The term "administered" means administration of a therapeutically or prophylactically effective dose of the cell lysate of the invention to an individual. By "therapeutically or prophylactically effective amount" is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art and described above, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

In accordance with the present invention the term "vaccination" is related to a general process for immunization against cancers, tumorous diseases, infections and/or autoimmune diseases. Vaccination is a form of deliberate artificial immunization whereby the cell lysates or with cell lysate loaded dendritic cells of the present invention are administered. The cell lysates are administered in a form as described herein, supra, and may sensitise the immune system such that if cancer, tumorous diseases, infections and/or autoimmune diseases arise within the body are being treated or prevented. See, for example, Immunology, at pp. 87-88; AMA Encyclopedia of Medicine at 573-574 and 1034; S. J. Cryz, Jr., in Immunotherapy and Vaccines, edited by Stanley J. Cryz, pp. 3-11, VCH, Weinheim, Germany 1991. For an overview of the immune system from a molecular perspective, see, for example, Mary S. Leffell, An Overview of the Immune System: The Molecular Basis for Immune Responses, in Human Immunology Handbook pp. 1-45. Vaccination is also associated with immunization.

Immunization is a general term, and the term vaccination is used when patients are immunized. In general, immunization can be used as a preventive or as a therapeutic treatment. The preventive use of immunization is a prophylactic treatment, whereas the use of immunization while the disease is in progress is immunotherapy. Immunization provides two types of acquired immunity, active and passive. Immunotherapy is the treatment of a disease by immunization, active or passive, or by the use of agents that modify the actions of lymphocytes. In particular, immunotherapy refers to the stimulation of the immune system and conventionally uses a form of immunostimulant, a substance that causes a general,

non-specific, stimulation of the immune system. The American Medical Association Encyclopedia of Medicine, p. 576 (this encyclopedia will hereinafter be referred to as "AMA Encyclopedia of Medicine").

In a method for inducing an immune response to treat or prevent cancer, tumorous diseases, infections and/or autoimmune diseases, one or more cell lysates or with cell lysate loaded dendritic cells according to the invention are provided, and an effective amount of one or more of the cell lysates or with cell lysate loaded dendritic cells are injected at least once so as to permit release of biologically active quantities of the immunostimulant over a period of time to induce an immune response to the presence of active tumor cells.

An individual for the purposes of the present invention includes both humans and other animals, preferably vertebrates and more preferably mammals. Thus the methods are applicable to both human therapy and veterinary applications. In a preferred embodiment the individual is a mammal, e.g. a mouse, and in a most preferred embodiment the individual is human.

The compounds described herein having the desired therapeutic or prophylactic activity may be administered in a physiologically acceptable carrier to a patient, as described herein. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways as discussed below. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt %. However, it is also envisaged that the person skilled in the art is readily in a position to determine the concentration of the therapeutically active compound in the formulation by using his common general knowledge. The agents maybe administered alone or in combination with other treatments, i.e., radiation, or other chemotherapeutic agents.

In a preferred embodiment, the pharmaceutical compositions are in a water-soluble form, such as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts.

The administration of the candidate agents of the present invention can be done in a variety of ways as discussed above, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intranodally, peritumorally, intratumorally, intrarectally, intraperitoneally, intramuscularly,

intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the candidate agents may be directly applied as a solution dry spray.

The cell lysates that are obtainable by the aforementioned processes can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal, intranodal, intrarectal, peritumotal, intratumoral or intrabronchial administration. The attending physician and clinical factors will determine the dosage regimen. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A typical dose of lysate can origin from about 1000 to 10^{13} cells and the typical dose for dendritic cells is about 10^4 to 10^{12} cells, however, doses below and above this exemplary range are envisaged. Preferably, the dose of lysate corresponds to amounts generated from cells numbers between 10^4 to 10^{12} cells, more preferably between 10^5 to 10^{11} cells, more preferably between 10^6 to 10^{10} cells. Preferably, the dose for loaded dendritic cells is between 10^5 to 10^{11} cells, more preferably between 10^6 to 10^9 cells. Doses can vary between individuals and can be split to multiple injections at different sites and/or administration routes. Suitable and optimal doses can be determined by those skilled in the art. The amount of cell lysate used for loading dendritic cells can be determined by those skilled in the art, for example by those in vitro and or in vivo assays which are exemplary shown in the examples. Preferable amounts for lysates originate from 10^3 to 10^{13} cells, more preamble from 10^4 to 10^{12} cells, more preferable from 10^5 to 10^{11} cells, and more preferably from 10^6 to 10^{10} cells. Generally, the regimen as a regular administration of the pharmaceutical or vaccine composition should be in the range of 0,1 µg to 10 g per dose for the lysates, preferably 50 to 100 mg, amounts for fractionated lysates can be correspondently lower but may reach the high amounts.. The dosages are preferably given once a week, however, during progression of the treatment the dosages can be given in much longer time intervals and in need can be given in much shorter time intervals, e.g., daily. In a preferred case the immune response is monitored using herein

described methods and further methods known to those skilled in the art and dosages are optimized, e.g., in time, amount and/or composition.

If the regimen is a continuous infusion, it should also be in the range of 0.1 µg to 10 mg per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. The cell lysates of the invention may be administered locally or systemically. Administration will preferably be parenterally, e.g., intravenously, intranodally, intra peritoneally, intra tumourally, peri tumorally. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions; including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

It is also envisaged that the cell lysates are employed in co-therapy approaches, i.e. in co-administration with other medicaments or drugs, for example anti-cancer drugs.

When vaccine therapy is carried out using the cell lysates or with cell lysate loaded dendritic cells of the present invention, they may be administered only once. However, it is desirable to repeat the administration to the same site of a body to achieve coexistence of a tumor antigen and a cytokine or a cytokine-inducing agent as long as possible. For example, both components may preferably coexist for 3 hours or more so that inflammatory reaction at the site of administration can be induced and conditions can be achieved wherein immune cells are concentrated and cells are kept at the site. When a cell lysate without adjuvant is administered, an adjuvant may be administered to the same site. Generally, the cell lysate can be administered to a patient from which the tumor material is derived; however, the vaccine can also be administered to a patient bearing a tumor that contains, from a

viewpoint of pathological diagnosis, the same or relative species of a tumor antigen as that contained in the tumor material. The site to be administered is not particularly limited. Preferred sites include those where cytokines are hardly be diffused and disappeared, for example, intradermal, subcutaneous or intramuscular sites, in lymphnodes, and in a main organ such as spleen. However, by choosing a dosage form, which prevents ready diffusion of the active ingredients of the tumor vaccine, local administrations may sometimes be performable to any site of a body, or by applying a drug delivery system, the systemic administration may sometimes be possible. The dose and administration period of the tumor vaccine of the present invention are not particularly limited. It is desirable to determine an appropriate dose and administration period by observing effects of the vaccine therapy. The administration can be made, for example, by injections and the like.

It is also preferred that the cell lysates or with cell lysate loaded dendritic cells are administered to an individual for the treatment or prevention of against infections, e.g. caused by microorganisms like bacteria, fungi, viruses and/or parasites or autoimmune diseases. An autoimmune disease may arise from immune recognition and reaction against the individual's own cells or parts of the own body. Another preferred embodiment for vaccination is a combination of an immunization with dendritic cells loaded a cell lysate according to the present invention followed by boosting with the necrotic cell lysate.

According to the present invention, the tumor cell lysates, which are used for the preparation of a pharmaceutical or vaccine composition, can either be autologous or allogeneic or xenogenic with respect to the treated individual. It is also envisaged that the tumor cell lysates can be obtained from a tumor/tumor cell material/metastasis and used for the preparation of a pharmaceutical or vaccine composition or cell lines, including cell lines, e.g., tumor cell lines, or primary cell lines or cell lines or cultures from primary material and the like administered for treating or preventing another kind of tumor. For example tumor cells derived from leukaemias or lymphomas can be used to treat or prevent colon carcinoma. In another preferred embodiment of the present invention the pharmaceutical or vaccine composition used for the vaccination comprises one or more tumor cell lysates produced from different tumors/tumor material/tumor cells in order to avoid a so-called tumor escape. These lysates can be loaded to dendritic cells or their

precursor states as described above and used as such as a component of vaccine or pharmaceutical compositions for administration.

Additionally, it is preferred that infected tumor cells as described hereinabove are used for vaccination against infections, whereby the infectious component harboured by the infected cells may be additionally inactivated by chemical or physical means. It is also envisaged that non-infectious variants or mutants of the infectious component are needed for infecting said tumor cells.

The invention also relates to the use of the lysate obtainable by the process according to the invention for the preparation of a vaccine or pharmaceutical composition for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases.

In a preferred embodiment the cancer or tumorous disease to be treated or prevented is a cancer/tumorous disease of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer. However, said cancer/tumorous disease may also be selected from those mentioned hereinabove in connection with the process according to the invention.

The infection to be treated or prevented is preferably bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection. More preferably the bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection is selected from the group consisting of bacterial infections like sepsis and sepsis shock, fever of unknown origin, infective endocarditis, intraabdominal infections and abscesses, acute infectious, diarrheal diseases and bacterial food poisoning, sexually transmitted diseases, pelvic inflammatory disease, urinary tract infections and pyelonephritis, osteomyelitis, infections of the skin, muscle, and soft tissues, infections in injection drug users,

Infections from bites, scratches, and burns, infections in transplant recipients and hospital-acquired and intravascular device-related infections. More preferably the infection to be treated or prevented is selected from the group consisting of bacterial infections like pneumococcal infections, staphylococcal infections, streptococcal and enterococcal infections, diphtheria, other corynebacterial infections and anthrax, listeria monocytogenes infections, clostridial infections, like tetanus, botulism, gas gangrene, antibiotic-assiociated colits, meningococcal infections, gonococcal infections, moraxella (branhamella) catarrhalis, other moraxella species, and kingella infections, Haemophilus infections caused by haemophilus species, the HACEK group and other gram-negative bacilli infections, legionella infections, pertussis infections, gram-negative enteric bacilli infections, helicobacter infections, pseudomonas species and related organisms infections, salmonella infections, shigella infections, campylobacter and related species infections, cholera and other vibrio infections, brucella infections, tularaemia infections, plague and other yersinia infections, bartonella infections, including cat-scratch disease, donovanosis (Granuloma Inguinale) infections, nocardiosis, actinomycosis, infections due to mixed anaerobic organism, tuberculosis, leprosy (Hanses's Disease), infections due to nontuberculous mycobacteria, syphilis, endemic treponematoses, leptospirosis, relapsing fever, lyme borreliosis, rickettsial diseases, mycoplasma infections, chlamydial infections, viral infections due to Herpes simple viruses, Varicella-zoster virus infections, Epstein-barr virus infections, including infectious mononucleosis, Cytomegalovirus and human herpesvirus types 6, 7, and 8 infections, smallpox, vaccinia, and other poxviruses infections, parvovirus inections, Human papillomavirus infections, common viral respiratory infections, influenzy, viral gastroenteritis, enteroviruses and reoviruses infections, measles, rubelly (German measles), mumps, rabies virus and other rhabdoviruses infections, infections caused by arthropod- and rodent-borne viruses, Marburg and ebola viruses (Filoviridae), fungal infections like histoplasmosis, coccidioidomycosis, blastomycosis, cryptococcosis, candidiasis, aspergillosis, mucormycosis, miscellaneous mycoses and prototheca infections, Pneumocystic carinii infection, protozoal infections like amebiasis and infection with free-living amebas, malaria and other diseases caused by red blood cell parasites, leishmaniasis, trypanosomiasis, Toxoplasma infection, protozoal intestinal infections and

trichomoniasis, helminthic infections, like trichinosis and infections with other tissue nematodes, Intestinal nematodes, filariasis and related infections (Loiasis, Onchocerciasis, and Dracunculiasis), schistosomiasis and other trematode infections or Cestodes infections.

The autoimmune disease to be treated or prevented is preferably selected from the group consisting of allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

In view of the in vivo and in vitro results of the examples of the present invention it is expected that the invention provides an advantageous cancer, tumorous disease, infection and/or autoimmune disease vaccine. In particular, mice developed both a humoral and cellular immune response when challenged with tumor antigens present in/on tumor cells, which served as a source for the produced cell lysate. Moreover, even antibodies against carbohydrate antigens have been developed which has not or rarely been observed so far when conventionally produced tumor cell lysates have been administered. Additionally, it was found that mice with an implemented human immune system showed the same phenomenon.

The Figures show:

Figure 1 Analysis of propidium iodide and annexin V-FITC labelled temperature induced NM-F9 tumor cells (A-C) and anti-Hsp70 labeled NM-F9 tumor cells by flow cytometry.

Figure 2 Detection of cellular Hsp 70 expression in temperature treated NM-F9 tumour cells derived from K562 tumor cells by immunocytochemical staining; panel a) shows NM-F9 cells incubated at 41.2°C, the staining of Hsp70 positive cells was done by using a secondary Cy3-labelled antibody; panel b) shows NM-F9 cells incubated at 46.2°C in the dark field control; panel c) shows the same NM-F9 cells incubated at 46.2°C, wherein Hsp70 positive cells are labeled with a secondary Cy3-labelld antibody

Figure 3 In vitro analysis of T cell (CD8) stimulation by temperature treated NM-F9 tumor cell lysate loaded dendritic cells

Figure 4 In vitro analysis of T cell (CD4) activation by mature dendritic cells which were incubated with different NM-F9 tumor lysates.

Figure 5 In vitro induction of CD8+ T-cell responses with various necrotic NM-F9 cell lysates after one stimulation.

The tables show:

Table 1 Induction of apoptosis, necrosis and expression of membrane bound HSP 70 protein on NM-F9 tumor cells derived from K562 by temperature treatment.

temperature	Percent of total (%)			
	cells	Apoptotic cells	Necrotic cells	Hsp 70 positive cells
37.0 °C	70 +/- 3.7	21 +/- 1.9	9 +/- 3.2	5
38.0 °C	64	25	9	1
40.0 °C	48	40	12	3
41.2 °C	44	40	15	33
42.0 °C	34	45	20	1
42.4 °C	25 +/- 10	59 +/- 11	16 +/- 0.35	1
43.0 °C	34	47	19	0.7
44.0 °C	25	52	23	10
46.2 °C	5 +/- 0.6	9 +/- 0.2	86 +/- 0.7	5

Table 2 Humoral immune response in NMRI mice induced by temperature pre-treated NM-F9 tumor cell lysates.

Vaccine	Number of mice with antigen specific IgG immune response/ total number			
	AGP	TF	Tn	MUC1
Necrotic tumor cell lysate 46.2 °C	2/3	3/3	3/3	3/3
Apoptotic tumor cell lysate 42.4 °C	2/3	0/3	0/3	1/3
Lysate of tumor cells with increased HSP70 expression 41.2 °C	0/3	0/3	0/3	0/3
Lysate of untreated tumor cells 37 °C	2/3	0/3	0/3	0/3

Table 3 Humoral immune response in NOD/SCID mice induced by temperature pre-treated NM-F9 tumor cells lysates.

Vaccine	Number of mice with antigen specific IgG immune response/total number			
	AGP	TF	Tn	MUC1
Necrotic tumor cell lysate 46.2 °C	3/3	3/3	3/3	3/3
Apoptotic tumor cell lysate 42.4°C	1/3	1/3	1/3	0/3
Lysate of tumor cells with increased HSP 70 expression 41.2 °C	0/3	0/3	0/3	0/3
Lysate of untreated cells 37 °C	0/3	1/3	1/3	0/3
Negative control ,Mel624 cell lysate, AGP-, TF-, Tn-, MUC1-	0/3	0/3	0/3	0/3

The examples illustrate the invention.

1. **Process of temperature treatment for preparation of apoptotic and necrotic tumor cell lysate**

In the following Example K562 cells (ATCC: CCL-243) may be used. However, preferably TF-positive F9 cells (NM-F9) derived from K562 tumor cells (ATCC: CCL-243) are used which were cultivated in RPMI media with 10% FCS, 1% glutamic acid (complete RPMI media), 8% CO_2, 95% humidity at 37°C. For each preparation $7.5x10^5$ K562-cells were harvested. Before temperature induction tumor cells were resuspended in 450 microliter complete RPMI media (for mouse vaccination, section 5) or in serum-free AIMV-media (for in vitro assay, section 4). For temperature induction four aliquots of 100 microliter and one aliquot of 50 microliter (0.5 ml e-cups) of the tumor cells were incubated for 2 h in a preheated thermocycler (Eppendorf, Hamburg, Germany). Afterwards temperature treated cells were resuspended in 25 ml complete RPMI media or AIMV media and recultured under conditions above (in complete RPMI media at 37°C). For induction of apoptosis and necrosis an incubation time of 22h found to be better than 6 h and 14.5 h (results described in section 2, flow cytometry). Before lysis, tumor cells were washed with PBS (for mouse vaccination) or AIMV (for in vitro assay) three times. Afterwards cell counts were adjusted to 5×10^6 cells/ 75 microliter PBS (for mouse vaccination) or 1×10^6 / 500 microliter AIMV (for in vitro assay). Cell lysis was performed by freezing in liquid nitrogen and thawing at 37°C (water bath) for three cycles (30 sec/phase in liquid nitrogen). Cells lysates were stored at –80°C.

To improve the immunogenity and to simplify the induction process for preparing necrotic cells, the duration of temperature induction (2h or 3h) as well as the reculture time at 37°C (0h, 3h, 22h) were varied. In these experiments temperature induction occurred in PBS or serum-free AIMV media in a thermoblock at 46.5°C. Heat-treated cells were recultured in the same volume and media in microcentrifuge tubes in which temperature induction had taken place. Necrotic cell lysate (in AIMV-media) was used for in vitro analysis (Figure 5). The following preparations were lysed by freezing and thawing: 1)

Temperature induction 2h (sample A) and 3h (sample B) without reculture time. 2) Temperature induction 2h (sample C) and 3h (sample D) and 3h reculture time. 3) Temperature induction 2h and 22h reculture time in microcentrifuge tubes (sample E). 4) as control: Temperature induction 2h and 22h reculture time resuspended media.

2. Determination of vital, apoptotic and necrotic cells by flow cytometry

For determination of vital, apoptotic and necrotic cells the Apoptosis Detection Kit I (Pharmingen, Heidelberg, Germany) containing Annexin V-FITC and Propidium iodide was used. Apoptotic cells were only stained by Annexin V-FITC, necrotic cells were stained by Annexin V-FITC and Propidium iodide (PI) . For analysis 3x 10^5 heat-treated and recultured K562 cells (ATCC: CCL-243) may be used. However, TF-positive F9 cells derived from K562 tumor cells (NM-F9) () were used which were harvested and washed in PBS twice. Cell pellets were resuspended in 1x binding buffer (10 mM Hepes pH 7.4, 140 mM NaCl and 2.5 mM CaCl 2) and stained with 5 microliter Annexin V-FITC and 2 microliter PI. After 15 min incubation time at room temperature (in a dark place) samples were diluted in 400 microliter 1x binding buffer. For flow cytometric analysis the flow cytometer Coulter Epics XL of Beckman-Coulter (Miami, USA) with Expo 32 ADC software (Beckman-Coulter) was used. For investigation the following parameter were used:
"sideward scatter": 807 volt, "gain" 5, forward scatter": 26 volt "gain" 1.
Annexin V: FL1-channel, 707 volt, "gain" 1, compensation FL1-FL3 0.4.
PI: FL3-channel, 729 volt, "gain" 1, compensation FL3-FL1 17.9.

Proportional determination of vital, necrotic and apoptotic cells of heat-treated 22h recultured TF-positive F9 cells derived from K562 cells (NM-F9) are described in "dot plots" diagrams in figure 1. K562-tumor cells were incubated in a thermocycler for 2h at 37°C (control, figure 1A), at 42.4°C (figure 1B) and at 46.2°C (figure 1C). After temperature induction cells were recultured 22h at 37°C.

In a "dot plot" diagram the fluorescence intensity of Annexin V-FITC/PI stained cells is described by the position of spots in the coordinate field. The coordinate field is separated into quadrants, whose position is determined by negative and positive control using of PI or Annexin V-FITC stained cells. The unstained population in the left quadrant below represents the vital cells (no dye can enter the cell), the population in the right quadrant below represents the apoptotic cells (only Annexin V-FITC can enter the cell) and the population in the upper left and right quadrant represents the necrotic cells.

To determine the best temperature for induction of apoptosis and necrosis, TF-positive F9 cells derived from K562-cells were incubated for 2h at 10 different temperatures described in table 1. As shown in figure 1B and C the optimal temperature for induction of apoptotic cells is 42.4°C and the temperature for induction of necrotic cells is 46.2°C.

3. Preparation of tumor cell lysate with high expression of membrane bound HSP 70 proteins

In the following Example K562 cells (ATCC: CCL-243) may be used. However, TF-positive F9 cells derived from K562 (NM-F9) are preferably used. With the latter cells it was observed that the preparation of heat-treated TF-positive F9 cells derived from K562 (NM-F9) tumor cell lysate with high expression of membrane bound HSP 70 is comparable to the preparation of necrotic and apoptotic cells. The optimal reculture time for TF-positive F9 cells derived from K562 cells (NM-F9) expressing high amounts of HSP 70 were 14.5h (results, section 4).

Detection of membrane bound HSP 70 protein by flow cytometry

3×10^5 TF-positive F9 cells derived from K562 cells (NM-F9) which are preferably used were harvested and resuspended in 25 microliter complete RPMI media. After 45 min incubation with anti-human HSP 70-IgG1 antibody (1:100 diluted in complete RPMI) at 4°C, cells were washed in PBS/10% FCS twice (5 min centrifugation at 1500 upm, Heraeus Multifuge). Afterwards the cells

were incubated for 30 min with a secondary Cy3-anti-mouse-IgG antibody at 4°C (1:200 diluted in PBS, Dianova, Hamburg, Germany). The cells were washed in PBS twice and resuspended in 200 microliter PBS. For analysis the following parameters were used: "sideward scatter" and "forward scatter" as already described.

Cy3: FL2-chanel, 740 volt, "gain" 1.

The results are described in the overlay-histogram (figure 1D), which show HSP 70 induction of the control (incubated at 37°C) and F9cells incubated at 41.2°C. As seen in the histogram in about 33% of the F9cell population incubated at 41.2°C the expression of HSP 70 proteins is increased.

The HSP 70 expression of TF-positive F9 cells derived from K562 cells (NM-F9) incubated for 2 h at 10 different temperatures is described in table 1. The optimal HSP 70 expression in heat-treated TF-positive F9 cells derived from K562 cells (NM-F9) is received by temperature induction at 41.2°C and 14.5h recultured at 37°C.

Detection of cellular HSP 70 expression by immunocytochemistry

The staining process occurred in a humid chamber.

For coating the slide 5 x 10^4 cells in 50 microliter were dropped on the slide (in a humid chamber), 30min incubated at 37°C in the CO_2 incubator and 1h incubated at room temperature. After removing the supernatant the samples were dried for 15 min at room temperature and stored at –20°C wrapped in aluminium foil. After thawing the cells were fixed in 5% formaldehyde (diluted in PBS, 5 min incubated at room temperature). The cells were washed in PBS three times and blocked by incubating 1 h with 5% BSA / PBS. After washing in PBS cells were labelled by anti-HSP 70 antibody (as above, 1:200 diluted in 1% BSA/PBS, 90 min incubation at RT). After washing the cells with PBS three times K562 cells were incubated for 1h with secondary Cy3- anti-mouse IgG antibody (1:200 diluted in 1% BSA/PBS) at room temperature. Finally the cells were embedded in Mowiol-solution (6g glycerin, 2.4g mowiol 4-88 (Calbiochem, Bad Soden, Germany), 6 ml H_2O, 12 ml 0.2 M Tris-HCl pH 8.5 mixing 2 h at RT,

15 min centrifugation at 5000xg, addition of Diazobizyclooctan (Sigma)). Immunochemical analysis was performed with a by Zeiss Microskope Axioplan (Oberkochen, Germany). The results of cellular HSP 70 – expression by F9 incubated at 41.2°C, incubated at 42.4°C (apoptotic cells) and incubated at 46.2°C (necrotic cells) is shown in figure 2. 80-90% of K562 cells incubated at 41.2°C have increased HSP 70 expression. In contrast 2-5% of necrotic cells incubated at 46.2°C showed increased HSP 70 expression.

For immunogenity studies lysates of four differently treated cell populations were prepared.
1) lysate of necrotic TF-positive F9 cells derived from K562 cells (NM-F9) (2 h temperature induction at 46.2°C, 22 h recultured at 37°C), 2) lysate of apoptotic TF-positive F9 cells derived from K562 cells (2 h temperature induction at 42.4°C, 22 h recultured at 37°C), 3) lysate of TF-positive F9 cells derived from K562 cells (NM-F9) with increased expression of HSP 70, (2 h temperature induction at 41.2°C, 14.5h recultured at 37°C), 4) lysate of untreated TF-positive F9 cells derived from K562 cells (incubated at 37°C).

4. Specific activation of CD8+ and CD4+ T-cells by heat treated tumor cell lysate taken up, processed and presented by dendritic cells

Dendritic cells can be incubated with K562 cells, however, are preferably incubated with different TF-positive F9 cells (NM-F9) derived from K562 tumor lysates overnight. After phagocytosis of TF-positive F9 cells derived from K562 lysates the maturation of dendritic cells was induced. Afterwards
the activation of CD8+ (figure 3) and CD4+ (figure 4) T-cells by mature dendritic cells was analysed and the dendritic cells were incubated with CD8+ and in order to stimulate these cells in an antigene-specific manner.

Preparation of immature human dendritic cells

Immature human dendritic cells were prepared by differentiation of human monocytes (hmoDC) by the method of Romani (Romani N et al. 1994, J. Exp.

Med. 180: 83-93). Peripheral blood monocytes were isolated from peripheral blood of healthy human donor by Ficoll gradient centrifugation. Adherent cells which adhere on plastic were cultured for 6 days in RPMI-1640, 10% FCS, 1000 U/ml GM-CSF 2.5ng/ml TNFα and 1000 U/ml IL-4 .
Secondly immature dendritic cells were prepared (Nemod-iDC, which are optimized MUTZ-3 derived immature dendritic cells as described in WO 03/023023 which can be purchased from NEMOD Immuntherapie AG, Robert-Rössle-Strasse 10, D-13125 Berlin, Germany)); from a human cell line according to the methods described in WO 03/023023

Loading and maturation of Nemod-iDC

The immature hmoDC or Nemod-iDC (10^6 cells/ sample) were incubated overnight
with tumor cell lysates at a proportion of 1:1. After washing the dendritic cells with sterile PBS GM-CSF, IL-4 and 75 ng/ml TNFα were added. The mature hmoDC, Nemod-mDC became CD14-, CD1a+, CD80hi, CD86hi, CD40hi, MHCIIhi, CD83hi, DC-Sign+ (flow cytometry, suffix : - = no expression, hi = high expression, + = positive expression). Prior to T cell sensitisation, the antigen loaded Nemod-mDC were irradiated with 30 Gy.

Preparation of peripheral blood T-cells

T cells were isolated from the non-adherent fraction of PBMC of healthy HLA-A2 positive donor by a column of nylon wool (Polysciences Inc., Eppelheim, Ger). Alternativaly CD4+ or CD8+ - T-cells were isolated from PBMC by CD4- or CD8-T-cell-MACS-Isolationkits (Miltenyi Biotec, Köln, Ger).

Induction of tumor cell lysate specific T-cells

Total T-cells, CD4+ or CD8+ T-cells were incubated in serum-free media (AIM-V medium) with mature hmoDC or Nemod-mDC loaded with cell lysate in serum. The ratio of responder : stimulator (T-cell : DC) was 10-20:1. After overnight incubation 10 U/ml IL-2, 1.5 U/ml IL-1β and 5ng/ml IL-7 were added. After

incubation for four days T-cells were restimulated by mature hmoDC or Nemod-mDC loaded with antigen. T-cells were analysed by IFNγ-ELISPOT Assay (figure 3 and 5) following overnight incubation or after 4 days (without addition of cytokine) the cell proliferation was analysed by BrdU assay (figure 4).

IFNγ-ELISPOT Assay

ELISPOT analysis was performed using a kit (Mabtech, Nacka, Sweden) and PVDF-bottomed 96-well-Multiscreen plates from Millipore (Bedford, USA). Before coating overnight with mouse-anti-human-IFNγ antibodies (15 microgram /ml, at 4°C) PVDF was soaked with 70% ethanol (50 microliter / well). After washing with PBS, $7x10^4$ T-cells together with the relevant number of antigen-loaded mature hmoDC or Nemod-mDC in 200 microliter medium / well were incubated 16h at 37°C. Cells were then removed and plates washed three times with PBS / Tween. Secreted IFNγ was detected by incubating with biotinylated anti-human-IFNγ antibody (50 microliter / well in PBS, 2h, RT), followed by a conjugate of streptavidin alkaline phosphatase (1:1000 dilution, 100 microliter / well, 1 h incubation at RT). After washing with PBS-Tween four times, detection of alkaline phosphatase was achieved by staining with BCIP (35 microliter / 10ml detection buffer) and NBT (45 microliter / 10ml detection buffer). The reaction was stopped by water. Prior to analysis with an ELISPOT Reader (Autoimmun Diagnostika GmbH, Strassberg, Germany) 96-well plates were dried for 1h at 40°C.

T-cell-proliferation assay (BrdU incorparation)

BrdU was incorporated into proliferating T cells in accordance with the manufacture's protocol. After fixing, the cells were incubated with POD labelled anti-BrdU- antibody. The subsequent staining reaction was stopped by 1M sulfuric acid. Detection of antibody labelling was achieved by photometry at an optical density of 450 nm (Ref. 690nm).

Results of in vitro analysis

In vitro experiments revealed that the immunogenity of tumor cell lysates was improved by heat pretreatment (figure 3, 4 and 5). The best results were achieved by temperature induction at 46.2°C (about 80% necrotic cells). This result was unexpected. In contrast to many authors preparing necrotic cells by lysing untreated cells (Gallucci, S. Nature Medicine 1999; Kotera, Y. Cancer Res. 2001; Restifo, N.P. Curr. Opin. Immunol. 2000; Sauter, B.J. et al. Exp. Med. 2000) we improved the immunogenity by temperature-induced necrosis of cells prior to lysis. In disagreement with other authors (Dressel (2000),loc. cit; Feng (2001), loc cit.; Melcher (1998), loc. cit; Todryk (2000),loc. cit.) we could not improve the immunogenity by increasing the expression of HSP 70 proteins (temperature induction at 41.2°C) (figure 3 and 4). As described in table 1 HSP 70 expression as determined by flow cytometry, was increased by temperature induction at 41.2°C. In both heat-induced necrotic cells (46.2°C) and apoptotic cells (42.4°C) the surface HSP 70 expression was reduced (necrotic cells) or unchanged (apoptotic cells) (table 1). These results were confirmed by immunofluorescence tests (figure 2, 2-5% of necrotic cells (46.2°C) HSP 70 positive, 80-90% of cells incubated at 41.2°C HSP 70 positive).

The highest cellular immunogenity (cytotoxic CD8+ T cells and CD4+ T helper cells) was achieved by temperature-induced necrotic cell lysates. Compared to lysates of untreated cells, cell lysates of temperature induced apoptotic cells also induced an improved CD8+ T cell immune response (figure 3). An improved cellular immune response was induced in the autologous system (hmoDC) as well as in the semi-allogeneic system (NemodDC). A strong immune response is based on CD8+ and CD4+ T cell activation. Therefore, development of tumor vaccines based on tumor cell lysates can be improved by using temperature-induced necrotic cell lysates. To simplify the process and to improve the immunogenity the preparation process of temperature-induced necrotic cells was optimised. All necrotic TF-positive F9 cells derived from K562 tumor cells (NM-F9) induced by a variety of preparation process (mentioned in section 1) show high immunogenity in "in vitro assay" (figure 5). Necrotic cell lysates induced by

2h incubation at 46.5°C without reculture (section 1, sample A) induced a small increase in CD8+ T-cell reaction.

5. Vaccination of mice by temperature treated human tumor cell lysine

a) "normal" NMRI mice

NMRI mice were vaccinated subcutaneously with lysates of temperature-treated tumor cells ($5x10^6$ cells/mouse)of K562 derived F9 cells (NM-F9). After two weeks mice were boosted with the same tumor vaccine. No tumor growth or other side effects were observed during the vaccination period. One day before immunisation and 9 and 27 days after immunisation mice were bled to analyse serum for TF-, Tn-, MUC 1- and asialoglycophorin A antibodies by ELISA. For these analyses, 96-well plates were coated with Thomsen-Friedenreich-disaccharide-polyacrylamide-conjugate (TF-PAA), Tn-monosaccharide-PAA-conjugate (Synthesome, Munich, Germany), asialoglycophorin A (AGPA, Sigma) (2 microgram/ml in 50 microliter PBS) and MUC 1 (diluted 1 : 40 in PBS, optimal dilution was determined by anti-MUC1-antibody A76-A/C7) purified from supernatant of tumor cells as described in PCT/EP03/08014 (After incubation overnight at 4°C 96-well plates were washed with 0.05% Tween 20 in PBS (washing buffer), blocked with 5 % BSA, 0.05 % Tween 20 in PBS (1.5 h incubation at RT) and washed again in washing buffer three times. The coated 96-well plates were incubated with different dilutions of mice sera for 2 h at room temperature. The purified antibodies A76-A/C7, A78-G/A7 (Cao (1997), Virchows Arch. 431, 159-166) and Tn-HB1 (diluted 1:500, Dako, Hamburg, Germany) were used as positive controls. For negative control the primary antibody was replaced by medium. After washing in washing buffer three times plates were incubated with peroxidase labelled anti-mouse IgG or IgM (isolated from rabbit, diluted 1:5000, Dianova). Finally plates were washed with washing buffer twice and with PBS one time. The ELISA was developed with 0.4 mg/ml o-phenyldiamine (Sigma) in 25mM citrate-phosphate buffer pH 5.0 with 0.04% H_2O_2 at room temperature (in the dark). The colour reaction was

stopped by addition of 2.5 N sulfuric acid (final concentration 0.07 N) and analysed by ELISA-reader at 490 nm (reference filter at 630 nm)

b) Specific activation of human IgG antibody immune response in NOD/SCID mouse model by vaccination of different temperature pre-treated tumor cell lysate

NOD-SCID mice provide a commonly used mouse model, popular because of their deficient immune system. In these mice the human immune system is established by intraperitoneal application of human peripheral blood lymphocytes into mice irradiated one day earlier (PBL, standard preparation, $5x10^7$ cell/mouse). 2-4 h after application of PBL, mice were vaccinated subcutaneously with tumor cell lysates of untreated, apoptotic, necrotic or HSP70 expressing cells as an emulsion with incomplete Freund' s adjuvant ($5x10^6$ cells/mouse, cells + adjuvant = 100 microliter). After 14 days mice were boosted by the same cell lysates. Cell lysates of Mel624 cells ($5x10^6$ cells/mouse) were used as negative control. There was no evidence of tumor growth or other side effects in NOD-SCID mice. For analysis of serum, mice were bled at days 13 and 28 after the first immunisation. The analyses were carried out by ELISA as described above, here the dilution of secondary POD labelled anti human IgG antibody was 1:10 000.

Results of in vivo analysis

A humoral IgG immune response against all tested antigens was induced in nearly all NMRI mice and NOD-SCID mice vaccinated by temperature induced necrotic cell lysate (tab. 2 and 3). An IgG immune response against TF and Tn carbohydrate antigen is unusual because immunisation of mice by TF-antigen usually induces an IgM immune response. This might indicate that activation of the immune response by temperature-induced cell lysates is superior immunisation process involving a switch of antibody class associated with a T helper cell immune response as well as induction of memory immune responses against the above antigens. A specific human

IgG immune response was induced in a murine immune system as well as in an implantated human immune system. Clearly cell lysates of necrotic cells induced a stronger specific humoral immune response than cell lysates of apoptotic cells. Cell lysates of tumor cells with increased HSP 70 expression (temperature induction at 41.2°C) did not induced a specific humoral immune response in either mouse model (tab. 2 and 3) . These are not in accordance with results of other authors (Dressel (2000), loc cit.; Feng (2001), loc. Cit.; Melcher, (1998), loc. cit.; Todryk (2000), loc. cit.) who described a strong immune response induced by cells with increased HSP 70 expression. Cell lysates of untreated tumor cells induced only poor specific humoral immune responses.

Claims

1. A process for the production of an immunogenic compound comprising the steps of
   (a) inducing necrosis by temperature in tumor cells; and
   (b) lysing said necrotic tumor cells so as to obtain a lysate.

2. The process of claim 1, wherein necrosis is induced in tumor cells selected from the group consisting of tumor cell lines, cells derived from primary tumor material, cells derived from cell populations of primary tumor material and/or metastases including micrometastases.

3. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 41.2°C.

4. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 42°C.

5. The process of any one of claims 1 to 4, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45°C to 55°C.

6. The process of any one of claims 1 to 5, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45.5°C to 47°C.

7. The process of any one of claims 1 to 6, wherein said induction of necrosis is for at least 15 minutes.

8. The process of any one of claims 1 to 7, wherein said induction of necrosis is performed in the range of 2 to 3 hours.

9. The process of any one of claims 1 to 8, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

10. The process of any one of claims 1 to 9, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

11. The process of any one of claims 1 to 10, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

12. The process of any one of claims 1 to 11, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

13. The process of any one of claims 1 to 11, wherein said tumor cells are allogeneic.

14. The process of any one of claims 1 to 11, wherein said tumor cells are syngenic.

15. The process of any one of claims 1 to 11, wherein said tumor cells are xenogenic.

16. The process of any one of claims 1 to 15, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

17. The process of any one of claims 1 to 16, wherein said tumor cells are NM-F9 cells (DSMZ deposit No._____) or NM-D4 cells (DSMZ deposit No._____).

18. A lysate obtainable by the process of any one of claims 1 to 17.

19. Dendritic cells loaded with the lysate of claim 18.

20. A composition comprising a lysate of claim 18 or dendritic cells of claim 19.

21. The composition of claim 20, which is a pharmaceutical composition.

22. The composition of claim 20, which is a vaccine composition.

23. The pharmaceutical composition of claim 21 or the vaccine composition of claim 22, which is optionally combined with an adjuvant.

24. The dendritic cells of claim 19 or the composition of claim 20, wherein said dendritic cells are immature.

25. The dendritic cells of claim 19 or the composition of claim 20, wherein said dendritic cells are mature.

26. A method for the production of a vaccine composition comprising the step of combining a cell lysate of claim 18 or the dendritic cells of claim 19 with an adjuvant.

27. A method for the production of a pharmaceutical composition comprising the step of combining a cell lysate of claim 18 with a pharmaceutically acceptable carrier.

28. A method for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases comprising administering a therapeutically or prophylactically effective amount of the lysate of claim 18 to an individual, or the pharmaceutical composition of claim 21, 23 or 24 or of the vaccine composition of any one of claims 22 to 24, or dendritc cells of claim 19.

29. Use of the lysate of claim 18 or of the dendritic cells of claim 19 for the preparation of a pharmaceutical or vaccine composition for the treatment or

prevention of cancers, tumorous diseases, infections and/or autoimmune diseases.

30. The method of claim 28 or the use of claim 29, wherein said cancer or tumorous disease is a cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer.

31. The method of claim 28 or the use of claim 29, wherein said infection is bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection.

32. The method of claim 28 or the use of claim 29, wherein said autoimmune disease is allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

Figure 1 A, B

Analysis of propidium iodide and FITC-AnnexinV labelled temperature induced tumor cells A-C and anti-Hsp70 labeled NM-F9 tumor cells by flow cytometry

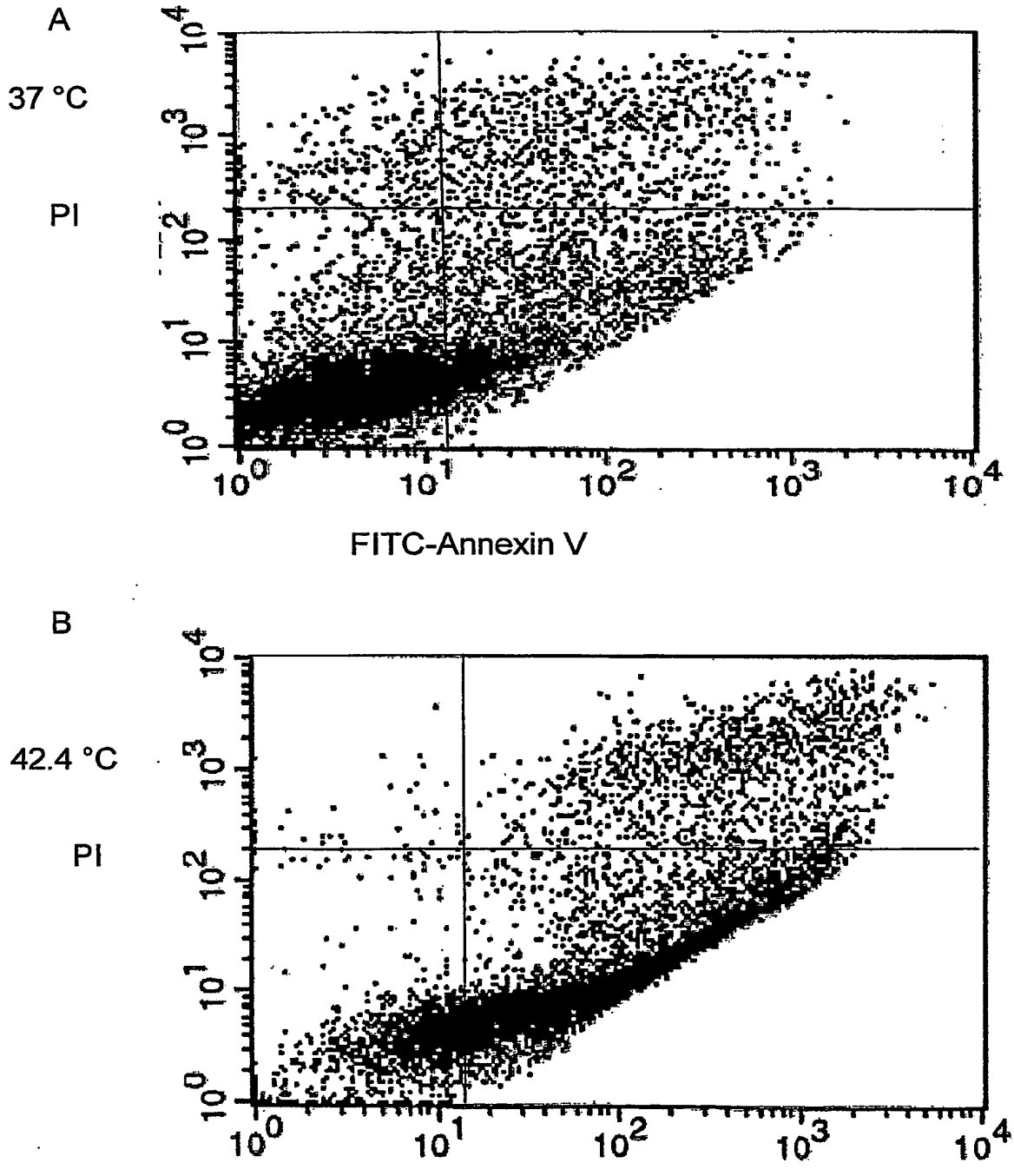


Figure 1 C

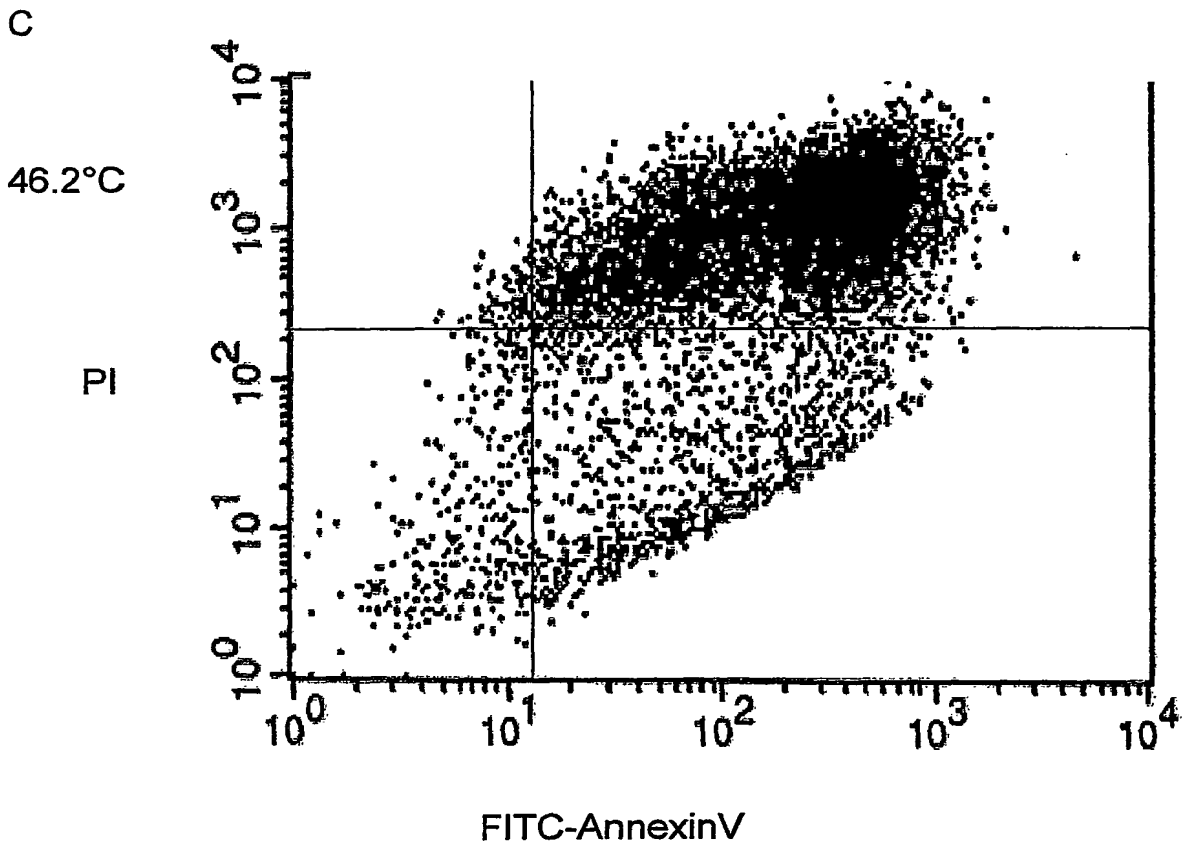


Figure 1 D

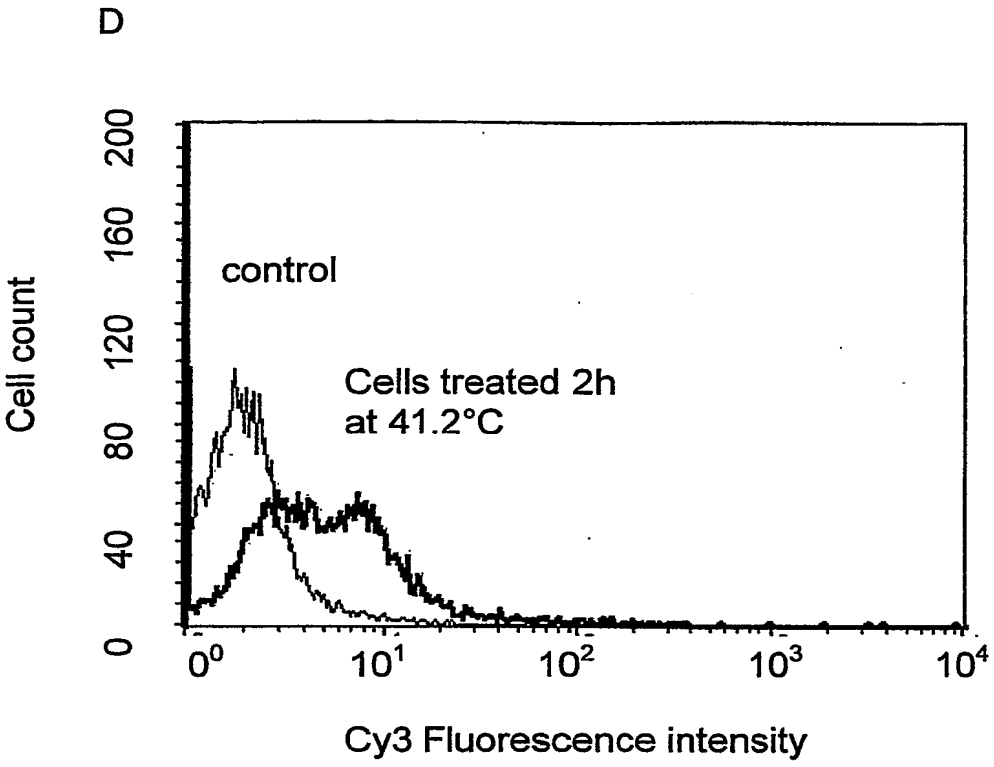
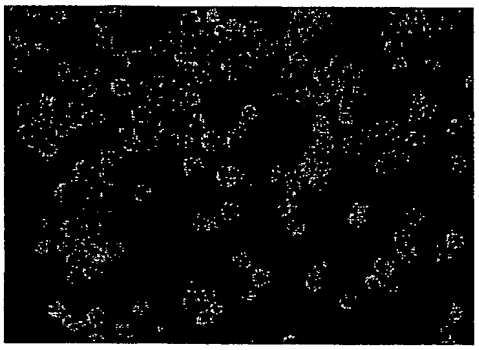

D
Cell count
0
40
80
120
160
200
control
Cells treated 2h
at 41.2°C
0^0
10^1
10^2
10^3
10^4
Cy3 Fluorescence intensity

Figure 2

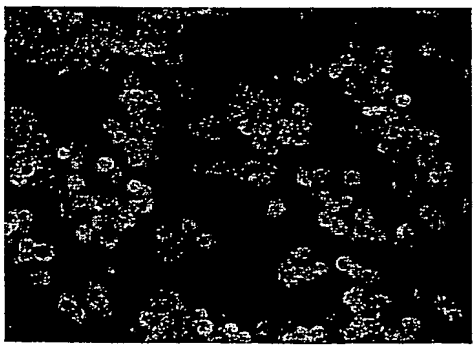
Detection of cellular Hsp 70 expression in temperature treated K562 tumor cells by immunocytochemical staining

NM-F9 derived from K562 cells incubated at 41.2°C

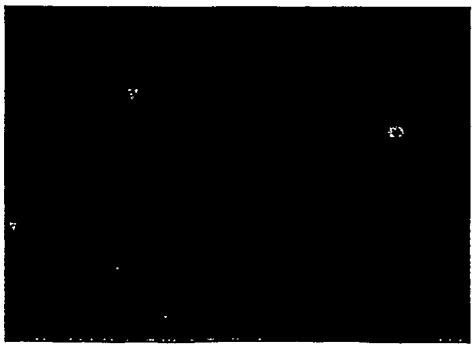


Hsp 70 positive NM-F9 cells stained with secondary Cy3-labelled antibody

NM-F9 derived from K562 cells incubated at 46.2°C



Dark field control



Hsp 70 positive NM-F9 cells stained with secondary Cy3-labelled antibody

Figure 3

In vitro analysis of T cell stimulation by temperature treated NM-F9 tumor cell lysate loaded dendritic cells

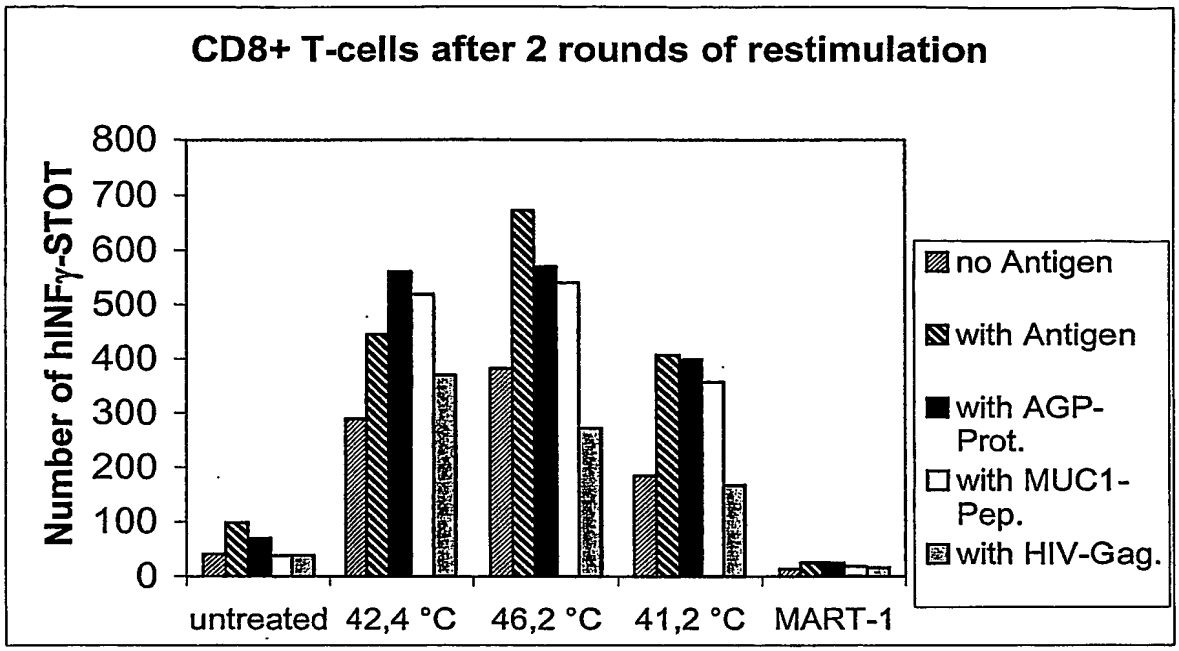
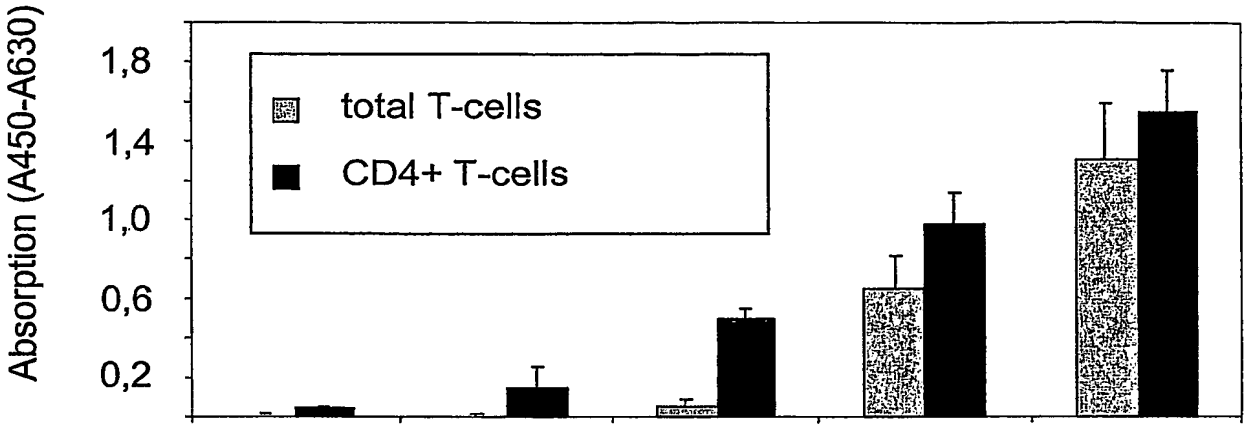


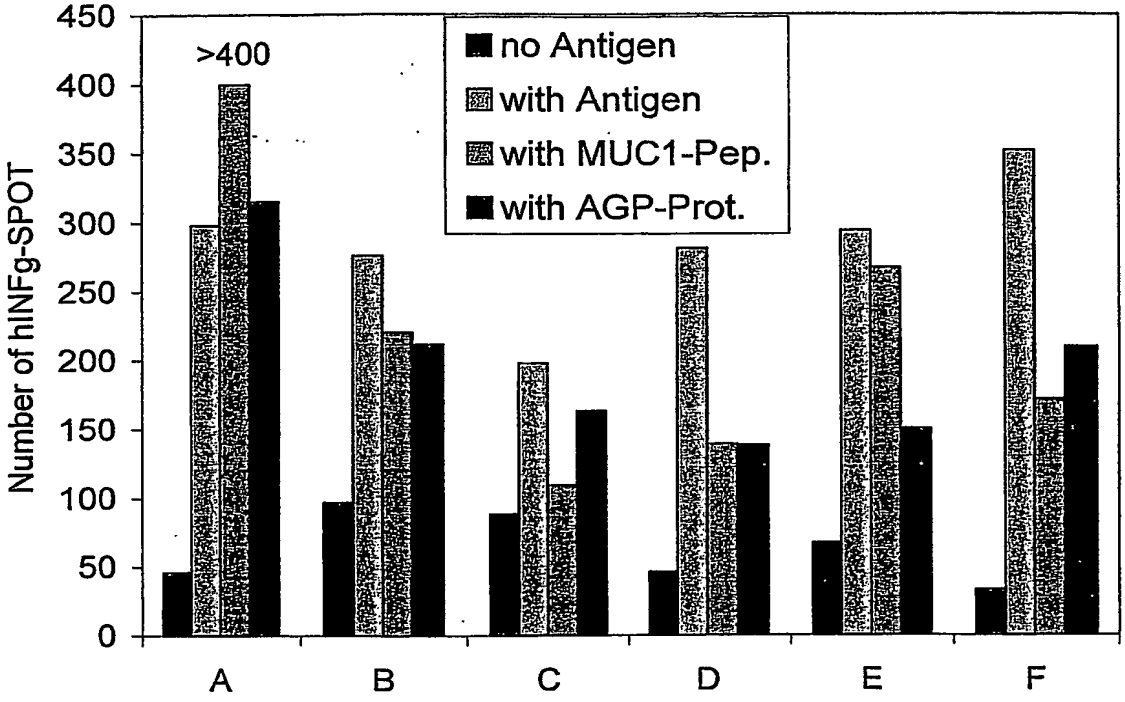
Figure 4



DC cells	+	-	+	+	+
T cells	-	+	+	+	+
Apoptotic tumor cell lysates	-	-	-	+	-
Necrotic tumor cell lysates	-	-	-	-	+

Figure 5

In vitro Induction of CD8+ T-cell responses with various necrotic NM-F9 cell lysates (after 1 Stimulation)



Necrotic NM-F9 lysates:

A) 2h 46.5 °C
B) 3h 46.5 °C
C) 2h 46.5 °C +
3h 37.0 °C
D) 3h 46.5 °C +
3h 37.0 °C
E) 2h 46.5 °C +
22 h 37 °C
F) 2h 46.5 °C +
22 h 37 °C

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

PCT/EP2003/009140

REC'D 17 NOV 2003

WIPO PCT

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2605

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only

☐ This sheet was received with the international application

Authorized officer

For International Bureau use only

☒ This sheet was received by the International Bureau on:
17 NOVEMBER 2003

Authorized officer
EKO

Form PCT/RO/134 (July1998)

CT/EP2003/009140

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NM-D4	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2605

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(x) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-08-14 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Wehlo Date: 2003-10-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

PCT/EP2003/009140

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

REC'D 17 NOV 2003
WIPO PCT

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Nemod Biotherapeutics GmbH & Co. KG Address: Robert-Rössle-Str. 10 13125 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2605 Date of the deposit or the transfer[1]: 2003-08-14

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-08-18 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Wehlis Date: 2003-10-16

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 12/2001

PCT/EP2003/009140

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

REC'D 17 NOV 2003
WIPO PCT

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2606

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only

☐ This sheet was received with the international application

Authorized officer

For International Bureau use only

☒ This sheet was received by the International Bureau on:
17 NOVEMBER 2003

Authorized officer
EKO

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

CT/EP2003/009140

DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

REC'D 17 NOV 2003
WIPO PCT

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NM-F9	DSM ACC2606

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(x) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-08-14 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH
Address:	Mascheroder Weg 1b D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

V. Wetz

Date: 2003-10-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001

CT/EP2003/009140

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

REC'D 17 NOV 2003
WIPO PCT

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Nemod Biotherapeutics GmbH & Co. KG Address: Robert-Rössle-Str. 10 13125 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2606 Date of the deposit or the transfer[1]: 2003-08-14

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-08-26 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss Date: 2003-10-16

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/09140

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/08 A61K39/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOMERSAN S ET AL: "Primary tumor tissue lysates are enriched in heat shock proteins and induce the maturation of human dendritic cells." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 NOV 2001, vol. 167, no. 9, 1 November 2001 (2001-11-01), pages 4844-4852, XP002267738 ISSN: 0022-1767 the whole document ----- -/--	1-32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2004	09/02/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN Z ET AL: "Efficient antitumor immunity derived from maturation of dendritic cells that had phagocytosed apoptotic/necrotic tumor cells." INTERNATIONAL JOURNAL OF CANCER. JOURNAL INTERNATIONAL DU CANCER. 15 AUG 2001, vol. 93, no. 4, 15 August 2001 (2001-08-15), pages 539-548, XP002267739 ISSN: 0020-7136 the whole document	1-32
A	KOTERA Y ET AL: "Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immunization." CANCER RESEARCH. 15 NOV 2001, vol. 61, no. 22, 15 November 2001 (2001-11-15), pages 8105-8109, XP002267740 ISSN: 0008-5472 the whole document	1-32
A	SAUTER BIRTHE ET AL: "Consequences of cell death: Exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 191, no. 3, 7 February 2000 (2000-02-07), pages 423-433, XP002267741 & ISSN: 0022-1007 the whole document	1-32
A	WO 99/29834 A (FORDHAM UNIVERSITY) 17 June 1999 (1999-06-17) cited in the application the whole document	1-32

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 03/09140

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 28 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/09140

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9929834 A	17-06-1999	US 5948646 A	07-09-1999
		AU 728036 B2	04-01-2001
		AU 1910699 A	28-06-1999
		CA 2314005 A1	17-06-1999
		EP 1037965 A1	27-09-2000
		JP 2001526025 T	18-12-2001
		WO 9929834 A1	17-06-1999
		US 6410026 B1	25-06-2002
		US 6406700 B1	18-06-2002
		US 6410027 B1	25-06-2002

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G2300 PCT S3	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09140	International filing date *(day/month/year)* 18.08.2003	Priority date *(day/month/year)* 16.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/08		
Applicant GLYCOTOPE GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.01.2004	20.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Halle, F Telephone No. +49 89 2399-8537

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09140

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21, 24-54	as originally filed
22, 23	received on 02.01.2004 with letter of 02.01.2004

Claims, Numbers

1-30	received on 08.11.2004 with letter of 08.11.2004

Drawings, Sheets

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15,17-30
	No:	Claims	16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-25,27
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Having considered the amended claims (allowable under Art. 34(2)(b) PCT) and the reply to the Written Opinion, the results of examination are as follows:

1. In this report, reference is made to the following documents:

   D1: Gough et al (2001), Cancer Research 61, 7240-7247
   (cited in the description; not cited in the international search report)

   D2: SOMERSAN S ET AL: "Primary tumour tissue lysates are enriched in heat shock proteins and induce the maturation of human dendritic cells." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 NOV 2001, vol. 167, no. 9, 1 November 2001 (2001-11-01), pages 4844-4852, XP002267738 ISSN: 0022-1767

   D3: KOTERA Y ET AL: "Comparative analysis of necrotic and apoptotic tumour cells as a source of antigen(s) in dendritic cell-based immunization." CANCER RESEARCH. 15 NOV 2001, vol. 61, no. 22, 15 November 2001 (2001-11-15), pages 8105-8109, XP002267740 ISSN: 0008-5472

2. The identification of the deposited tumour cells by the deposit numbers (DSM ACC2605 and ACC2606) in the description (pages 22 and 23) and in claim 15 are in principle allowable and the basis therefor will be the publication of the indication relating to microorganisms, i.e. PCT-Form RO/134 together with the specification (PCT Guidelines 4.19).

3. The invention relates to the production of immunogenic compounds for the vaccination against cancers, the immunogenic compounds being obtained from necrotic tumour cells where the necrosis of these cells has been induced by temperature.

   Having regard to the above cited prior art, the subject-matter of claims 1-15 appears, in principle, to be novel (Article 33(2) PCT) but does not appear to involve an inventive step (Article 33(3) PCT).

D1 refers to freeze-thawing and heat shock of tumour cells. D2 and D3 also refer to freeze-thawing of cells in order to obtain necrotic cells. Taking into account that heat-shock and other temperature dependent methods are already disclosed in the prior art for the induction of necrosis of tumor cells, the formulation of said claims appears to be a trivial formulation based on routine features; the skilled person would select such routine features, in accordance with circumstances, without the exercise of inventive skill, in order to obtain immunogenic compounds against cancer cells.
In particular, in the proposed process claimed, necrosis of the tumor cells by temperature is a mandatory technical feature in order to obtain immunogenic tumor cells. However, the induction of necrosis by temperature is known, see heat shock of tumor cells in D1, p.7241, left column, paragraph "Freeze-Thawing and Heat Shock of Tumor Cells". The other feature involved in the process claimed is the lysing of the cells; but this feature is not necessary for the induction of necrosis and thus not essentiel for the desired effect, i.e. the immunogenicity of the tumor cells. Therefore, lysing the cells may help to render the claimed process formally novel but not inventive over the known necrosis inducing temperature dependent methods (D1-D3).

4. Lysates are generally known in the state of the art (see also D1-D3). Since claim 16 does not contain any technical feature which would allow to clearly distinguish the lysate claimed from prior art lysates, the subject-matter of claim 16 cannot be considered as novel or as involving an inventive step.

5. The subject-matter of the product claims 17-23 may be considered as formally novel. Nevertheless, due to the definition of said claims in general terms, and having regard to the fact that the lysate is not defined (any lysate known or cited in D1-D3 may be concerned), the formulation of said claims is obvious to the skilled person; said claims therefore do not involve an inventive step.
This remark also applies to claims 24-30.

6. For the assessment of the present claims 26 and 28-30 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a

known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

7. The above cited prior art documents should be mentioned in the description (Rule 5.1(a)(ii) PCT) (if not already done).

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
Germany

Date of mailing (day/month/year) 16 January 2004 (16.01.2004)	
Applicant's or agent's file reference G2300 PCT S3	**IMPORTANT NOTIFICATION**
International application No. PCT/EP2003/009140	International filing date (day/month/year) 18 August 2003 (18.08.2003)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 16 August 2002 (16.08.2002)
Applicant GLYCOTOPE GMBH et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. (If applicable) The **letters "NR"** appearing in the right-hand column denote **a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. (If applicable) An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes **a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
16 Augu 2002 (16.08.2002)	02018512.0	EP	16 Janu 2004 (16.01.2004)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.70

Authorized officer

Peter WIMMER (Fax 338 8970)

Telephone No. (41-22) 338 9896

PATENT COOPERATION TREATY Rec'd PCT/PTO 16 FEB 2005

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
ALLEMAGNE

EINGEGANGEN
Vossius & Partner
15. März 2004
Frist bearb.:

Date of mailing *(day/month/year)* 04 March 2004 (04.03.2004)	
Applicant's or agent's file reference G2300 PCT S3	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2003/009140	18 August 2003 (18.08.2003)	16 August 2002 (16.08.2002)

Applicant

GLYCOTOPE GMBH et al

1. Notice is hereby given that the International Bureau has **communicated**, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

   AU, AZ, BY, CH, CN, CO, DZ, EP, HU, JP, KG, KP, KR, MD, MK, MZ, RU, TM, US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CR, CU, CZ, DE, DK, DM, EA, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PG, PH, PL, PT, RO, SC, SD, SE, SG, SK, SL, SY, TJ, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-*bis*)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 04 March 2004 (04.03.2004) under No. WO 2004/018659

4. **TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase**

   The applicable time limit for entering the national phase will, **subject to what is said in the following paragraph,** be **30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of **19 months** from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

   In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For **regular updates on the applicable time limits** (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   For filing a **demand for international preliminary examination**, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

   It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.(41-22) 740.14.35

Authorized officer

Gijsbertus Beijer - Carlos Roy

Telephone No.(41-22) 338.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
Germany

Date of mailing (day/month/year) 05 December 2003 (05.12.03)	
Applicant's or agent's file reference G2300 PCT S3	**IMPORTANT NOTIFICATION**
International application No. PCT/EP03/09140	**International filing date** (day/month/year) 18 August 2003 (18.08.03)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
NEMOD IMMUNTHERAPIE AG Robert-Rössle-Str. 10 13125 Berlin Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
GLYCOTOPE GMBH Robert-Rössle-Str. 10 13125 Berlin Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
Assignment.

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Elisabeth KÖNIG (Fax 338 8970)
Facsimile No. (41-22) 338.89.70	Telephone No. (41-22) 338 8748

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* G2300 PCT S3

Box No. I TITLE OF INVENTION

Process for the production of temperature-induced tumor cell lysates for use as immunogenic compounds

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

NEMOD Immuntherapie AG
Robert-Rössle-Str. 10
13125 Berlin
DE

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GOLETZ, Steffen
Eichhornstrasse 24
16548 Glienicke
DE

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Vossius & Partner
Siebertstraße 4
81675 Munich
Germany

Telephone No.
+49 89 41 30 40

Facsimile No.
+49 89 41 30 4111

Teleprinter No.

Agent's registration No. with the Office

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BAUMEISTER, Hans
Hochsitzweg 159
14169 Berlin
DE

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SCHÖBER, Ute
Kreuzstr. 7
13187 Berlin
DE

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☒ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

☒ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** AT Austria, BE Belgium, BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, HU Hungary, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, RO Romania, SE Sweden, SI Slovenia, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates	☒ HR Croatia	☒ OM Oman
☒ AG Antigua and Barbuda	☒ HU Hungary	☒ PG Papua New Guinea
☒ AL Albania	☒ ID Indonesia	☒ PH Philippines
☒ AM Armenia	☒ IL Israel	☒ PL Poland
☒ AT Austria	☒ IN India	☒ PT Portugal
☒ AU Australia	☒ IS Iceland	☒ RO Romania
☒ AZ Azerbaijan	☒ JP Japan	☒ RU Russian Federation
☒ BA Bosnia and Herzegovina	☒ KE Kenya	
☒ BB Barbados	☒ KG Kyrgyzstan	☒ SC Seychelles
☒ BG Bulgaria	☒ KP Democratic People's Republic of Korea	☒ SD Sudan
☒ BR Brazil		☒ SE Sweden
☒ BY Belarus	☒ KR Republic of Korea	☒ SG Singapore
☒ BZ Belize	☒ KZ Kazakhstan	☒ SK Slovakia
☒ CA Canada	☒ LC Saint Lucia	☒ SL Sierra Leone
☒ CH & LI Switzerland and Liechtenstein	☒ LK Sri Lanka	☒ SY Syrian Arab Republic
☒ CN China	☒ LR Liberia	☒ TJ Tajikistan
☒ CO Colombia	☒ LS Lesotho	☒ TM Turkmenistan
☒ CR Costa Rica	☒ LT Lithuania	☒ TN Tunisia
☒ CU Cuba	☒ LU Luxembourg	☒ TR Turkey
☒ CZ Czech Republic	☒ LV Latvia	☒ TT Trinidad and Tobago
☒ DE Germany	☒ MA Morocco	
☒ DK Denmark	☒ MD Republic of Moldova	☒ TZ United Republic of Tanzania
☒ DM Dominica		☒ UA Ukraine
☒ DZ Algeria	☒ MG Madagascar	☒ UG Uganda
☒ EC Ecuador	☒ MK The former Yugoslav Republic of Macedonia	☒ US United States of America
☒ EE Estonia		
☒ ES Spain	☒ MN Mongolia	☒ UZ Uzbekistan
☒ FI Finland	☒ MW Malawi	☒ VC Saint Vincent and the Grenadines
☒ GB United Kingdom	☒ MX Mexico	☒ VN Viet Nam
☒ GD Grenada	☒ MZ Mozambique	☒ YU Serbia and Montenegro
☒ GE Georgia	☒ NI Nicaragua	☒ ZA South Africa
☒ GH Ghana	☒ NO Norway	☒ ZM Zambia
☒ GM Gambia	☒ NZ New Zealand	☒ ZW Zimbabwe

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ ..... ☐ ..... ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 16 August 2002 (16/08/02)	02 01 8512.0		EP	
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☐ all items ☒ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* . . . .

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following **declarations** are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*: Number of declarations

☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application **contains**:

(a) **in paper form**, the following number of sheets :

request (including declaration sheets)	:	5
description (excluding sequence listings and/or tables related thereto)	:	54
claims	:	4
abstract	:	1
drawings	:	7
Sub-total number of sheets	:	71
sequence listings	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)		
Total number of sheets	:	71

(b) ☐ **only in computer readable form** (Section 801(a)(i))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

(c) ☐ **also in computer readable form** (Section 801(a)(ii))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listings: . . . . . .
- ☐ tables related thereto: . . . . . .

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)

This international application is **accompanied by** the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*: — Number of items

1. ☐ fee calculation sheet :
2. ☐ original separate power of attorney :
3. ☐ original general power of attorney :
4. ☐ copy of general power of attorney; reference number, if any: . . . . . :
5. ☐ statement explaining lack of signature :
6. ☐ priority document(s) identified in Box No. VI as item(s): . . . . . :
7. ☐ translation of international application into *(language)*: . . . . . :
8. ☐ separate indications concerning deposited microorganism or other biological material :
9. ☐ sequence listings in computer readable form *(indicate type and number of carriers)*
   - (i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application) :
   - (ii) ☐ *(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter* :
   - (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listings mentioned in left column :
10. ☐ tables in computer readable form related to sequence listings *(indicate type and number of carriers)*
    - (i) ☐ copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application) :
    - (ii) ☐ *(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*) :
    - (iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column :
11. ☐ other *(specify)*: . . . . . :

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

F. Stolzenburg

Dr. Friederike Stolzenburg
European Patent Attorney

Vossius & Partner
Siebertstr. 4
81675 München
(Nr. 31)

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: G2300 PCT S3

Applicant: NEMOD Immuntherapie AG

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . EUR 100.00 [T]

2. SEARCH FEE . . . . . EUR 945.00 [S]

International search to be carried out by EPO

(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

Where items (b) and/or (c) of Box No. IX apply, enter **Sub-total number of sheets** / Where items (b) and (c) of Box No. IX do not apply, enter **Total number of sheets**: 71

[b1] first 30 sheets . . . . . EUR 444.00 [b1]

[b2] 41 (number of sheets in excess of 30) x 10.00 (fee per sheet) = EUR 410.00 [b2]

[b3] additional component (only if sequence listings and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii)):

400 x 10.00 (fee per sheet) = EUR [b3]

Add amounts entered at b1, b2 and b3 and enter total at B . . . . EUR 854.00 [B]

Designation Fees

The international application contains all designations.

5 (number of designation fees payable *(maximum 5)*) x 96.00 (amount of designation fee) = EUR 480.00 [D]

Add amounts entered at B and D and enter total at I . . . . . EUR 1,334.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . EUR 30.00 [P]

5. TOTAL FEES PAYABLE . . . . . EUR 2,409.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☒ authorization to charge deposit account (see below) ☐ postal money order ☐ cash ☐ coupons

☐ cheque ☐ bank draft ☐ revenue stamps ☐ other *(specify)*:

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

Receiving Office: RO/ EPO

Deposit Account No.: 2800.0321

Date: September 16, 2003

Name: Dr. Friederike Stolzenburg

Signature: F. Stolzenburg

☒ Authorization to charge the total fees indicated above.

☒ *(This check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

☒ Authorization to charge the fee for priority document.

Claims

1. A process for the production of an immunogenic compound comprising the steps of
   (a) inducing necrosis by temperature in tumor cells; and
   (b) lysing said necrotic tumor cells so as to obtain a lysate.

2. The process of claim 1, wherein necrosis is induced in tumor cells selected from the group consisting of tumor cell lines, cells derived from primary tumor material, cells derived from cell populations of primary tumor material and/or metastases including micrometastases.

3. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 41.2°C.

4. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 42°C.

5. The process of any one of claims 1 to 4, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45°C to 55°C.

6. The process of any one of claims 1 to 5, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45.5°C to 47°C.

7. The process of any one of claims 1 to 6, wherein said induction of necrosis is for at least 15 minutes.

8. The process of any one of claims 1 to 7, wherein said induction of necrosis is performed in the range of 2 to 3 hours.

9. The process of any one of claims 1 to 8, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

10. The process of any one of claims 1 to 9, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

11. The process of any one of claims 1 to 10, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

12. The process of any one of claims 1 to 11, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

13. The process of any one of claims 1 to 11, wherein said tumor cells are allogeneic.

14. The process of any one of claims 1 to 11, wherein said tumor cells are syngenic.

15. The process of any one of claims 1 to 11, wherein said tumor cells are xenogenic.

16. The process of any one of claims 1 to 15, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

17. The process of any one of claims 1 to 16, wherein said tumor cells are NM-F9 cells (DSMZ deposit No._____) or NM-D4 cells (DSMZ deposit No._____).

18. A lysate obtainable by the process of any one of claims 1 to 17.

19. Dendritic cells loaded with the lysate of claim 18.

20. A composition comprising a lysate of claim 18 or dendritic cells of claim 19.

21. The composition of claim 20, which is a pharmaceutical composition.

22. The composition of claim 20, which is a vaccine composition.

23. The pharmaceutical composition of claim 21 or the vaccine composition of claim 22, which is optionally combined with an adjuvant.

24. The dendritic cells of claim 19 or the composition of claim 20, wherein said dendritic cells are immature.

25. The dendritic cells of claim 19 or the composition of claim 20, wherein said dendritic cells are mature.

26. A method for the production of a vaccine composition comprising the step of combining a cell lysate of claim 18 or the dendritic cells of claim 19 with an adjuvant.

27. A method for the production of a pharmaceutical composition comprising the step of combining a cell lysate of claim 18 with a pharmaceutically acceptable carrier.

28. A method for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases comprising administering a therapeutically or prophylactically effective amount of the lysate of claim 18 to an individual, or the pharmaceutical composition of claim 21, 23 or 24 or of the vaccine composition of any one of claims 22 to 24, or dendritc cells of claim 19.

29. Use of the lysate of claim 18 or of the dendritic cells of claim 19 for the preparation of a pharmaceutical or vaccine composition for the treatment or

prevention of cancers, tumorous diseases, infections and/or autoimmune diseases.

30. The method of claim 28 or the use of claim 29, wherein said cancer or tumorous disease is a cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer.

31. The method of claim 28 or the use of claim 29, wherein said infection is bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection.

32. The method of claim 28 or the use of claim 29, wherein said autoimmune disease is allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

immunogenic compounds by inducing necrosis in the tumor cells and subsequently lysing the cells. The obtained tumor cell lysates can be used for the therapeutic or prophylactic treatment of cancer, tumorous diseases, infections and /or autoimmune diseases.

In this contex the term "immunogenic compound" means compounds having the ability to evoke immune reactions of the cells of the immune system like macrophages, dendritic cells, Langerhans' cells, B- (B1 and B2) and/or T-cells (cytotoxic T cells (Tc), T-helper cells (Th0, Th1 and Th2)), natural killer cells (NK cells), memory cells and the like Preferably, the term "immunogenic compound" means compounds having the ability to evoke humoral and/or cellular immune response, wherein at least one of the cells/group of cells of immune effector cells or cell products of said immune effector cells, for example one or more of the aforementioned cells are involved. The person skilled in the art is aware of various methods to determine whether an immune response is evoked. Examples for methods used for this purpose are shown in the examples and be transferred, where necessary by those skilled in the art, to determine the response in an individual or patient treated with the lysates according to the invention. Furthermore, other methods known to those skilled in the art can complement these techniques.

In general, an immunogenic compound leads to an immune response comprising humoral and/or cellular responses; normally comprising that genes or gene products that affect the level of immune responses are expressed/activated, e.g. those of the major histocompatibility class (MHC) I and II, those of antibody light and heavy chains, those of members of the immunglobulin superfamily, those of T-cell receptor/receptor compounds, those of cytokines or those of signal transduction cascades involved in transmitting immune responses.

In a preferred embodiment, the tumor cells used for the production of a cell lysate as described herein are NM-F9 (DSMZ deposit No.____) or NM-D4 cells (DSMZ deposit No. ____).

The term "NM-F9" (also referred herein as "F9" or "TF-positive F9 cells") or "NM-D4" means cell lines or cells derived from the human myelogenous leukemia cell line K562 (ATCC: CCL-243). NM-F9 and NM-D4 were deposited with the Deutsche

Sammlung für Mikroorganismen und Zellkulturen GmbH ("DSMZ") on _____, 2003. The DSMZ is located at the Mascheroder Weg 1b, D-38124 Braunschweig, Germany. The DSMZ deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

The present invention also relates to a lysate obtainable or obtained by the process according to the present invention and to dendritic cells loaded with such a lysate.

Moreover, the present invention also relates to a composition comprising a lysate or dendritic cells according to the present invention.

In a preferred embodiment said composition is a pharmaceutical composition. In accordance with the present invention the term "pharmaceutical composition " relates to compositions comprising the cell lysates described hereinabove which are obtained by the aforementioned processes and having the desired pharmacological activity. Such pharmaceutical compositions comprise a therapeutically effective amount of the cell lysates of the present invention, and a pharmaceutically acceptable carrier. The pharmaceutical composition may be administered with a physiologically acceptable carrier to a patient, as described herein. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018659 A1

(51) International Patent Classification[7]: C12N 5/08, A61K 39/00, A61P 35/00

(21) International Application Number: PCT/EP2003/009140

(22) International Filing Date: 18 August 2003 (18.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02018512.0 16 August 2002 (16.08.2002) EP

(71) Applicant *(for all designated States except US)*: **GLYCOTOPE GMBH** [DE/DE]; Robert-Rössle-Str. 10, 13125 Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GOLETZ, Steffen** [DE/DE]; Eichhornstrasse 24, 16548 Glienicke (DE). **BAUMEISTER, Hans** [DE/DE]; Hochsitzweg 159, 14169 Berlin (DE). **SCHÖBER, Ute** [DE/DE]; Kreuzstr. 7, 13187 Berlin (DE).

(74) Agent: **VOSSIUS & PARTNER**; Siebertstrasse 4, 81675 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/018659 A1

(54) Title: PROCESS FOR THE PRODUCTION OF TEMPERATURE-INDUCED TUMOR CELL LYSATES FOR USE AS IMMUNOGENIC COMPOUNDS

(57) Abstract: The present invention relates to a process for the production of an immunogenic compound comprising inducing necrosis by temperature in tumor cells and lysing said necrotic tumor cells so as to obtain a lysate. Furthermore, the invention provides a method for the production of a pharmaceutical composition. Additionally, the invention relates to a pharmaceutical composition comprising a lysate obtainable by the aforementioned process. Moreover, methods and uses for vaccination against cancers, tumorous diseases, infections and/or autoimmune diseases comprising administering the cell lysates of the invention or dendritic cells loaded with the cell lysate loaded to an individual are provided.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/[redacted]03/09140

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/08 A61K39/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOMERSAN S ET AL: "Primary tumor tissue lysates are enriched in heat shock proteins and induce the maturation of human dendritic cells." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 NOV 2001, vol. 167, no. 9, 1 November 2001 (2001-11-01), pages 4844-4852, XP002267738 ISSN: 0022-1767 the whole document -/--	1-32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2004	09/02/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern[redacted] Application No
PCT[redacted] 03/09140

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN Z ET AL: "Efficient antitumor immunity derived from maturation of dendritic cells that had phagocytosed apoptotic/necrotic tumor cells." INTERNATIONAL JOURNAL OF CANCER. JOURNAL INTERNATIONAL DU CANCER. 15 AUG 2001, vol. 93, no. 4, 15 August 2001 (2001-08-15), pages 539-548, XP002267739 ISSN: 0020-7136 the whole document	1-32
A	KOTERA Y ET AL: "Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immunization." CANCER RESEARCH. 15 NOV 2001, vol. 61, no. 22, 15 November 2001 (2001-11-15), pages 8105-8109, XP002267740 ISSN: 0008-5472 the whole document	1-32
A	SAUTER BIRTHE ET AL: "Consequences of cell death: Exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 191, no. 3, 7 February 2000 (2000-02-07), pages 423-433, XP002267741 & ISSN: 0022-1007 the whole document	1-32
A	WO 99/29834 A (FORDHAM UNIVERSITY) 17 June 1999 (1999-06-17) cited in the application the whole document	1-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/09140

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 28 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC[redacted] 03/09140

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9929834 A	17-06-1999	US 5948646 A	07-09-1999
		AU 728036 B2	04-01-2001
		AU 1910699 A	28-06-1999
		CA 2314005 A1	17-06-1999
		EP 1037965 A1	27-09-2000
		JP 2001526025 T	18-12-2001
		WO 9929834 A1	17-06-1999
		US 6410026 B1	25-06-2002
		US 6406700 B1	18-06-2002
		US 6410027 B1	25-06-2002

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EPO

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/EP03/09140	18/08/03	16/08/02

Title of invention
"Process for the production of temperature-induced tumor cell lysates for us as immunogenic compounds"

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GLYCOTOPE GMBH
Robert-Rössle-Str. 10
13125 Berlin
DE

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GOLETZ, Steffen
Eichhornstrasse 24
16548 Glienicke
DE

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BAUMEISTER, Hans
Hochsitzweg 159
14169 Berlin
DE

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

[X] Further applicants are indicated on a continuation sheet.

Sheet No. 2.

International application No.
PCT/EP03/09140

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SCHÖBER, Ute
Kreuzstr. 7
13187 Berlin
DE

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/EP03/09140

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Vossius & Partner
Siebertstr. 4
81675 Munich
GERMANY

Telephone No.
0049 89 41 30 40

Facsimile No.
0049 89 41 30 4111

Teleprinter No.

Agent's registration No. with the Office

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[ ] the international application as originally filed

the description [ ] as originally filed
[X] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[X] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 4.

International application No.
PCT/EP03/09140

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

			For International Preliminary Examining Authority use only	
			received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	3 sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate power of attorney
3. ☐ original general power of attorney
4. ☐ copy of general power of attorney; reference number, if any:
5. ☐ statement explaining lack of signature
6. ☐ sequence listings in computer readable form
7. ☐ tables in computer readable form related to sequence listings
8. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Dr. Hans-Rainer Jaenichen
European Patent Attorney

Vossius & Partner
Siebertstr. 4
81675 Munich

(No. 31)

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/EP03/09140	For International Preliminary Examining Authority use only
Applicant's or agent's file reference	Date stamp of the IPEA

Applicant
GLYCOTOPE GMBH

CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee	EUR 1,530.00 P	
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	EUR 159.00 H	
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	EUR 1,689.00 TOTAL	

MODE OF PAYMENT

☒	authorization to charge deposit account with the IPEA (see below)	☐	cash
☐	cheque	☐	revenue stamps
☐	postal money order	☐	coupons
☐	bank draft	☐	other *(specify)*:

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all IPEAs)

IPEA/ EPO

☒ Authorization to charge the total fees indicated above.

☒ *(This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

Deposit Account No.: 2800.0321

Date: December 30, 2003

Name: Dr. Hans-Rainer Jaenichen

Signature:

PCT/EP03/09140
NEMOD Immuntherapie AG
Our Ref.: G2300 PCT S3

ADDITIONAL SHEET

Amendment under Article 34 PCT:

In compliance with Rule 13*bis* PCT, in particular, Rule 13*bis*4(a)(i) we have timely furnished to the International Bureau according to Rule 13*bis* 3(iii) to (iv) the date of deposit of the biological material deposited in connection with the present application, the accession number given to the deposit by the respective instruction and any further additional matter.

Accordingly, we now enclose substitute pages 22, 23 and 56. The substitute pages contain the date of deposit and the accession number of the deposited biological material. Basis for the amendment will be the publication of the indication relating to microorganisms, i.e. PCT-Form RO/134 together with the specification.

Thus, we submit the amendment made does not introduce subject matter which is beyond the disclosure of the published PCT application and, accordingly, complies with the provisions of Article 34(2)(b)PCT.

Dr. Hans-Rainer Jaenichen
European Patent Attorney

Enclosure:
Substitute pages 22, 23 and 56, in triplicate

immunogenic compounds by inducing necrosis in the tumor cells and subsequently lysing the cells. The obtained tumor cell lysates can be used for the therapeutic or prophylactic treatment of cancer, tumorous diseases, infections and /or autoimmune diseases.

In this contex the term "immunogenic compound" means compounds having the ability to evoke immune reactions of the cells of the immune system like macrophages, dendritic cells, Langerhans' cells, B- (B1 and B2) and/or T-cells (cytotoxic T cells (Tc), T-helper cells (Th0, Th1 and Th2)), natural killer cells (NK cells), memory cells and the like Preferably, the term "immunogenic compound" means compounds having the ability to evoke humoral and/or cellular immune response, wherein at least one of the cells/group of cells of immune effector cells or cell products of said immune effector cells, for example one or more of the aforementioned cells are involved. The person skilled in the art is aware of various methods to determine whether an immune response is evoked. Examples for methods used for this purpose are shown in the examples and be transferred, where necessary by those skilled in the art, to determine the response in an individual or patient treated with the lysates according to the invention. Furthermore, other methods known to those skilled in the art can complement these techniques.

In general, an immunogenic compound leads to an immune response comprising humoral and/or cellular responses, normally comprising that genes or gene products that affect the level of immune responses are expressed/activated, e.g. those of the major histocompatibility class (MHC) I and II, those of antibody light and heavy chains, those of members of the immunglobulin superfamily, those of T-cell receptor/receptor compounds, those of cytokines or those of signal transduction cascades involved in transmitting immune responses.

In a preferred embodiment, the tumor cells used for the production of a cell lysate as described herein are NM-F9 (DSMZ deposit No. DSM ACC2606 or NM-D4 cells (DSMZ deposit No. DSM ACC2605)

The term "NM-F9" (also referred herein as "F9" or "TF-positive F9 cells") or "NM-D4" means cell lines or cells derived from the human myelogenous leukemia cell line K562 (ATCC: CCL-243). NM-F9 and NM-D4 were deposited with the Deutsche

Sammlung für Mikroorganismen und Zellkulturen GmbH ("DSMZ") on August 14, 2003. The DSMZ is located at the Mascheroder Weg 1b, D-38124 Braunschweig, Germany. The DSMZ deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

The present invention also relates to a lysate obtainable or obtained by the process according to the present invention and to dendritic cells loaded with such a lysate.

Moreover, the present invention also relates to a composition comprising a lysate or dendritic cells according to the present invention.

In a preferred embodiment said composition is a pharmaceutical composition. In accordance with the present invention the term "pharmaceutical composition " relates to compositions comprising the cell lysates described hereinabove which are obtained by the aforementioned processes and having the desired pharmacological activity. Such pharmaceutical compositions comprise a therapeutically effective amount of the cell lysates of the present invention, and a pharmaceutically acceptable carrier. The pharmaceutical composition may be administered with a physiologically acceptable carrier to a patient, as described herein. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also

9. The process of any one of claims 1 to 8, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

10. The process of any one of claims 1 to 9, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

11. The process of any one of claims 1 to 10, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

12. The process of any one of claims 1 to 11, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

13. The process of any one of claims 1 to 11, wherein said tumor cells are allogeneic.

14. The process of any one of claims 1 to 11, wherein said tumor cells are syngenic.

15. The process of any one of claims 1 to 11, wherein said tumor cells are xenogenic.

16. The process of any one of claims 1 to 15, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

17. The process of any one of claims 1 to 16, wherein said tumor cells are NM-F9 cells (DSMZ deposit No. DSM ACC2606) or NM-D4 cells (DSMZ deposit No. DSM ACC2605).

18. A lysate obtainable by the process of any one of claims 1 to 17.

19. Dendritic cells loaded with the lysate of claim 18.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
ALLEMAGNE

PCT

WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	25.08.2004
Applicant's or agent's file reference G2300 PCT S3	**REPLY DUE within 2 month(s) and 15 days** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/09140	18.08.2003	16.08.2002

International Patent Classification (IPC) or both national classification and IPC
C12N5/08

Applicant
GLYCOTOPE GMBH et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 16.12.2004

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer
Halle, F

Formalities officer (incl. extension of time limits)
Rauf, A
Telephone No. +49 89 2399-7548

I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-21, 24-54	as originally filed
22, 23	received on 02.01.2004 with letter of 02.01.2004

Claims, Numbers

1-8, 20-32	as originally filed
9-19	received on 02.01.2004 with letter of 02.01.2004

Drawings, Sheets

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: __, which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1,2,18-20,26-29
Inventive step (IS)	Claims	1-32
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

Re Item V

1. Reference is made to the following document/s/:

D1: Gough et al (2001), Cancer Research 61, 7240-7247
(cited in the description; not cited in the international search report)

D2: SOMERSAN S ET AL: "Primary tumour tissue lysates are enriched in heat shock proteins and induce the maturation of human dendritic cells." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 NOV 2001, vol. 167, no. 9, 1 November 2001 (2001-11-01), pages 4844-4852, XP002267738 ISSN: 0022-1767

D3: KOTERA Y ET AL: "Comparative analysis of necrotic and apoptotic tumour cells as a source of antigen(s) in dendritic cell-based immunization." CANCER RESEARCH. 15 NOV 2001, vol. 61, no. 22, 15 November 2001 (2001-11-15), pages 8105-8109, XP002267740 ISSN: 0008-5472

Amendments (identification of deposited cells)

2.1 The amended data of the deposited tumour cells (description and claims submitted on 02.01.2004) is in principle allowable since (as stated by the Applicant in his letter) the basis therefor will be the publication of the indication relating to microorganisms (i.e. PCT-Form RO/134) together with the specification (see also PCT Guidelines 4.19).
The Applicant is kindly asked to eventually submit a copy of said Form RO/134.

Novelty, inventive step, definition of the invention

2.2 The invention relates to the production of immunogenic compounds for the vaccination against cancers, the immunogenic compounds being obtained from necrotic tumour cells where the necrosis of these cells has been induced by temperature.

2.3 Having regard to the cited prior art, the subject-matter of claims 1 and 2 does not

appear to be novel (Article 33(2) PCT) or to involve an inventive step (Article 33(3) PCT).

D1 refers to freeze-thawing and heat shock of tumour cells, see "Materials and Methods", page 7241, left column, paragraph "Freeze-Thawing and Heat Shock of Tumour Cells". D2 and D3 also refer to freeze-thawing of cells in order to obtain necrotic cells. The process of claims 1 and 2 which is defined by broad features like "inducing necrosis by temperature in tumour cells" (see claim 1(a)) obviously embraces all techniques involving the temperature: freezing and thawing, heat shock...etc. Therefore, the subject-matter of claims 1 and 2 is considered to embrace the cited prior art.

2.4 Claims 1 and 2 do not appear to contain the (essential) technical features (Article 6 and Rule 6.3(a) PCT) which should define the invention and therefore allow to unambiguously distinguish the invention from the prior art.

The features already mentioned above like "inducing necrosis by temperature" (claim 1(a)) without any further precision of what is meant by "temperature" are insufficient to define the present invention. It therefore appears necessary to introduce features in the claims whereupon not only novelty but also inventive step should be based in order to acknowledge for example a "surprising" effect or any advantage of the process of the invention over the cited prior art.

The above objections under items 2.2-2.4 also apply to the independent claims 18, 19, 20, 26, 27, 28 and 29 relating directly or indirectly to claims 1 and 2.

2.5 Having regard to the cited prior art, the subject-matter of the remaining claims 3-17, 21-25 and 30-32 appears, in principle, to be novel, but does not appear to involve an inventive step.

Taking into account that heat-shock and other temperature dependent methods are already known (see above items 2.2-2.4) for the induction of necrosis of tumor cells, the formulation of said claims appears to be a trivial formulation based on routine features; the skilled person would select such routine features, in accordance with circumstances, without the exercise of inventive skill, in order to obtain

immmunogenic compounds against cancer cells.

Industrial application

3.1 No objection is made against industrial application of claims 1-27 and 29.

3.2 For the assessment of the present claims 28 and 30-32 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Further comments and/or requirements

4. The above cited prior art documents should be mentioned in the description (Rule 5.1(a)(ii) PCT) (if not already done).

5. In the case of amendments, the Applicant is requested to file them by way of replacement pages in the manner stipulated by Rule 66.8(a) PCT. In particular, fair copies of the amendments should be filed preferably in triplicate. Moreover, the applicant's attention is drawn to the fact that, as a consequence of Rule 66.8(a) PCT the examiner is not permitted to carry out any amendments under the PCT procedure, however minor these may be.

In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the Applicant is requested to clearly identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see also Rule 66.8(a) PCT). If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed.

Any information the Applicant may wish to submit concerning the subject-matter of

the invention, for example further details of its advantages or of the problem it solves, and for which there is no basis in the application as filed, should be confined to the letter of reply rather than be incorporated into the application, Article 34(2)(b) PCT.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
ALLEMAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	20.12.2004
Applicant's or agent's file reference G2300 PCT S3	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/09140	18.08.2003	16.08.2002

Applicant
GLYCOTOPE GMBH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Rauf, A. Tel. +49 89 2399-7548

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G2300 PCT S3	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09140	International filing date *(day/month/year)* 18.08.2003	Priority date *(day/month/year)* 16.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/08		
Applicant GLYCOTOPE GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.01.2004	20.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Halle, F Telephone No. +49 89 2399-8537

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09140

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21, 24-54	as originally filed
22, 23	received on 02.01.2004 with letter of 02.01.2004

Claims, Numbers

1-30	received on 08.11.2004 with letter of 08.11.2004

Drawings, Sheets

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15,17-30
	No:	Claims	16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-25,27
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Having considered the amended claims (allowable under Art. 34(2)(b) PCT) and the reply to the Written Opinion, the results of examination are as follows:

1. In this report, reference is made to the following documents:

   D1: Gough et al (2001), Cancer Research 61, 7240-7247
   (cited in the description; not cited in the international search report)

   D2: SOMERSAN S ET AL: "Primary tumour tissue lysates are enriched in heat shock proteins and induce the maturation of human dendritic cells." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 NOV 2001, vol. 167, no. 9, 1 November 2001 (2001-11-01), pages 4844-4852, XP002267738 ISSN: 0022-1767

   D3: KOTERA Y ET AL: "Comparative analysis of necrotic and apoptotic tumour cells as a source of antigen(s) in dendritic cell-based immunization." CANCER RESEARCH. 15 NOV 2001, vol. 61, no. 22, 15 November 2001 (2001-11-15), pages 8105-8109, XP002267740 ISSN: 0008-5472

2. The identification of the deposited tumour cells by the deposit numbers (DSM ACC2605 and ACC2606) in the description (pages 22 and 23) and in claim 15 are in principle allowable and the basis therefor will be the publication of the indication relating to microorganisms, i.e. PCT-Form RO/134 together with the specification (PCT Guidelines 4.19).

3. The invention relates to the production of immunogenic compounds for the vaccination against cancers, the immunogenic compounds being obtained from necrotic tumour cells where the necrosis of these cells has been induced by temperature.

   Having regard to the above cited prior art, the subject-matter of claims 1-15 appears, in principle, to be novel (Article 33(2) PCT) but does not appear to involve an inventive step (Article 33(3) PCT).

D1 refers to freeze-thawing and heat shock of tumour cells. D2 and D3 also refer to freeze-thawing of cells in order to obtain necrotic cells. Taking into account that heat-shock and other temperature dependent methods are already disclosed in the prior art for the induction of necrosis of tumor cells, the formulation of said claims appears to be a trivial formulation based on routine features; the skilled person would select such routine features, in accordance with circumstances, without the exercise of inventive skill, in order to obtain immunogenic compounds against cancer cells.
In particular, in the proposed process claimed, necrosis of the tumor cells by temperature is a mandatory technical feature in order to obtain immunogenic tumor cells. However, the induction of necrosis by temperature is known, see heat shock of tumor cells in D1, p.7241, left column, paragraph "Freeze-Thawing and Heat Shock of Tumor Cells". The other feature involved in the process claimed is the lysing of the cells; but this feature is not necessary for the induction of necrosis and thus not essentiel for the desired effect, i.e. the immunogenicity of the tumor cells. Therefore, lysing the cells may help to render the claimed process formally novel but not inventive over the known necrosis inducing temperature dependent methods (D1-D3).

4. Lysates are generally known in the state of the art (see also D1-D3). Since claim 16 does not contain any technical feature which would allow to clearly distinguish the lysate claimed from prior art lysates, the subject-matter of claim 16 cannot be considered as novel or as involving an inventive step.

5. The subject-matter of the product claims 17-23 may be considered as formally novel. Nevertheless, due to the definition of said claims in general terms, and having regard to the fact that the lysate is not defined (any lysate known or cited in D1-D3 may be concerned), the formulation of said claims is obvious to the skilled person; said claims therefore do not involve an inventive step.
This remark also applies to claims 24-30.

6. For the assessment of the present claims 26 and 28-30 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a

known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

7. The above cited prior art documents should be mentioned in the description (Rule 5.1(a)(ii) PCT) (if not already done).

CLAIMS

1. A process for the production of an immunogenic compound comprising the steps of
   (a) inducing necrosis by a temperature of more than 41.2°C for at least 15 minutes in tumor cells; and
   (b) lysing said necrotic tumor cells so as to obtain a lysate.

2. The process of claim 1, wherein necrosis is induced in tumor cells selected from the group consisting of tumor cell lines, cells derived from primary tumor material, cells derived from cell populations of primary tumor material and/or metastases including micrometastases.

3. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 42°C.

4. The process of any one of claims 1 to 3, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45°C to 55°C.

5. The process of any one of claims 1 to 4, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45.5°C to 47°C.

6. The process of any one of claims 1 to 5, wherein said induction of necrosis is performed in the range of 2 to 3 hours.

7. The process of any one of claims 1 to 6, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

8. The process of any one of claims 1 to 7, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

9. The process of any one of claims 1 to 8, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

10. The process of any one of claims 1 to 9, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

11. The process of any one of claims 1 to 9, wherein said tumor cells are allogeneic.

12. The process of any one of claims 1 to 9, wherein said tumor cells are syngenic.

13. The process of any one of claims 1 to 9, wherein said tumor cells are xenogenic.

14. The process of any one of claims 1 to 13, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

15. The process of any one of claims 1 to 14, wherein said tumor cells are NM-F9 cells (DSMZ deposit No DSM ACCC2606) or NM-D4 cells (DSMZ deposit No. DSM ACC2605).

16. A lysate obtainable by the process of any one of claims 1 to 15.

17. Dendritic cells loaded with the lysate of claim 16.

18. A composition comprising a lysate of claim 16 or dendritic cells of claim 17.

19. The composition of claim 18, which is a pharmaceutical composition.

20. The composition of claim 18, which is a vaccine composition.

21. The pharmaceutical composition of claim 20 or the vaccine composition of claim 20, which is optionally combined with an adjuvant.

22. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are immature.

23. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are mature.

24. A method for the production of a vaccine composition comprising the step of combining a cell lysate of claim 16 or the dendritic cells of claim 17 with an adjuvant.

25. A method for the production of a pharmaceutical composition comprising the step of combining a cell lysate of claim 16 with a pharmaceutically acceptable carrier.

26. A method for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases comprising administering a therapeutically or prophylactically effective amount of the lysate of claim 16 to an individual, or the pharmaceutical composition of claim 19, 21 or 22 or of the vaccine composition of any one of claims 20 to 22, or dendritc cells of claim 16.

27. Use of the lysate of claim 16 or of the dendritic cells of claim 17 for the preparation of a pharmaceutical or vaccine composition for the treatment or prevention of cancers, tumorous diseases, infections and/or autoimmune diseases.

28. The method of claim 26 or the use of claim 27, wherein said cancer or

tumorous disease is a cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer.

29. The method of claim 26 or the use of claim 27, wherein said infection is bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection.

30. The method of claim 26 or the use of claim 27, wherein said autoimmune disease is allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

immunogenic compounds by inducing necrosis in the tumor cells and subsequently lysing the cells. The obtained tumor cell lysates can be used for the therapeutic or prophylactic treatment of cancer, tumorous diseases, infections and /or autoimmune diseases.

In this contex the term "immunogenic compound" means compounds having the ability to evoke immune reactions of the cells of the immune system like macrophages, dendritic cells, Langerhans' cells, B- (B1 and B2) and/or T-cells (cytotoxic T cells (Tc), T-helper cells (Th0, Th1 and Th2)), natural killer cells (NK cells), memory cells and the like Preferably, the term "immunogenic compound" means compounds having the ability to evoke humoral and/or cellular immune response, wherein at least one of the cells/group of cells of immune effector cells or cell products of said immune effector cells, for example one or more of the aforementioned cells are involved. The person skilled in the art is aware of various methods to determine whether an immune response is evoked. Examples for methods used for this purpose are shown in the examples and be transferred, where necessary by those skilled in the art, to determine the response in an individual or patient treated with the lysates according to the invention. Furthermore, other methods known to those skilled in the art can complement these techniques.

In general, an immunogenic compound leads to an immune response comprising humoral and/or cellular responses, normally comprising that genes or gene products that affect the level of immune responses are expressed/activated, e.g. those of the major histocompatibility class (MHC) I and II, those of antibody light and heavy chains, those of members of the immunglobulin superfamily, those of T-cell receptor/receptor compounds, those of cytokines or those of signal transduction cascades involved in transmitting immune responses.

In a preferred embodiment, the tumor cells used for the production of a cell lysate as described herein are NM-F9 (DSMZ deposit No. DSM ACC2606 or NM-D4 cells (DSMZ deposit No. DSM ACC2605)

The term "NM-F9" (also referred herein as "F9" or "TF-positive F9 cells") or "NM-D4" means cell lines or cells derived from the human myelogenous leukemia cell line K562 (ATCC: CCL-243). NM-F9 and NM-D4 were deposited with the Deutsche

Sammlung für Mikroorganismen und Zellkulturen GmbH ("DSMZ") on August 14, 2003. The DSMZ is located at the Mascheroder Weg 1b, D-38124 Braunschweig, Germany. The DSMZ deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

The present invention also relates to a lysate obtainable or obtained by the process according to the present invention and to dendritic cells loaded with such a lysate.

Moreover, the present invention also relates to a composition comprising a lysate or dendritic cells according to the present invention.

In a preferred embodiment said composition is a pharmaceutical composition. In accordance with the present invention the term "pharmaceutical composition " relates to compositions comprising the cell lysates described hereinabove which are obtained by the aforementioned processes and having the desired pharmacological activity. Such pharmaceutical compositions comprise a therapeutically effective amount of the cell lysates of the present invention, and a pharmaceutically acceptable carrier. The pharmaceutical composition may be administered with a physiologically acceptable carrier to a patient, as described herein. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also

EPA / EPO / OEB
D - 80298 München
089 / 2399 - 0
Tx 523 656 epmu d
Fax 089 / 2399 - 4465

Europäisches Patentamt | European Patent Office | Office européen des brevets

EPA / EPO / OEB : D-80298 München
Vossius & Partner
Siebertstr. 4

81675 München

Nr. der Anmeldung / Application No. / Demande de brevet no PCT/EP 0 3 / 0 9 1 4 0
Tag des Eingangs / Date of receipt / Date de réception **18.08.2003**
Zeichen des Anmelders / Vertreter - Applicant / Representative ref. no. - Référence du demandeur ou du mandataire **G2300 PCT S3**

Anmelder / Applicant / Demandeur : **Nemod Immuntherapie AG** Datum / Date **19.08.03**

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

Das Europäische Patentamt bescheinigt hiermit den Empfang folgender Dokumente:
The European Patent Office hereby acknowledges the receipt of the following:
L'Office européen des brevets accuse réception des documents indiqués ci-dessous:

A. **Internationale** Anmeldung / **International** application / Demande **internationale**

Stückzahl / No.of copies / Nombre d'exemplaires

- ☒ Antrag / Request / Requête — 1
- ☒ Beschreibung (ohne Sequenzprotokollteil) Description (excluding sequence listing part) Description (sauf partie réservée au listage des séquences) — 3
- ☒ Patentansprüche / Claim(s) / Revendication(s) — 3
- ☒ Zusammenfassung / Abstract / Abrégé — 3
- ☒ Zeichnung(en) / Drawing(s) / Dessin(s) — 3
- ☐ Sequenzprotokollteil der Beschreibung Sequence listing part of description Partie de la description réservée au listage des séquences — ____
- ☐ Diskette / Disquette — ____

- Kopie der allgemeinen Vollmacht / Copy of general power of attorney / Copie du pouvoir général
- Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité
- ☐ Blatt für die Gebührenberechnung / Fee calculation sheet / Feuille de calcul des taxes
- ☐ Abbuchungsauftrag / Debit order / Ordre de débit — Währung/Currency/Monnaie / Betrag/Amount/Montant
- ☐ Scheck / Cheque / Chèques — Ausfüllung freigestellt / Optional / facultatif
- ☐ Sonstige Unterlagen (einzeln aufführen) / Other documents (specify) / Autres documents (préciser)

B. Beigefügte Dokumente / Accompanying documents / Eléments joints

Gesonderte unterzeichnete Vollmacht / Separate signed power of attorney / Pouvoir distinct signé

Die genannten Unterlagen sind am oben genannten Tag eingegangen. Die in der Kontrolliste (Feld VIII) des PCT-Antragformulars RO/101 angegebenen Blattzahlen wurden bei Eingang nicht geprüft. Die Anmeldung hat ebenfalls oben angeführte Anmeldenummer erhalten / The said items were received on the date indicated above. No check was made on receipt that the number of sheets indicated in the check list (box VIII) of the PCT Request Form RO/101 were correct. The application has been assigned the above-indicated application number / Les documents mentionnées ont été reçus à la date indiquée. L'exactitude du nombre de feuilles indiqué au bordereau (cadre VIII) du formulaire de requête PCT RO/101 n'a pas été contrôlée lors du dépot. Le numéro figurant ci-dessus a été attribué à la demande de brevet.

Unterschrift / Amtsstempel / Signature / Official Stamp / Signature / Cachet officiel

Europäisches Patentamt
European Patent Office
Office européen des brevets
D-80298 München
A. Sandkühler

VOSSIUS & PARTNER

Vossius & Partner POB 86 07 67 81634 Munich Germany

European Patent Office
Erhardtstr. 27

80298 M Ü N C H E N

PCT/EP03/09140
Glycotope GmbH
Our Ref.: G2300 PCT S3

November 8, 2004
JAE/GW/ABE

This is in response to the Written Opinion of August 25, 2004 issued by the IPEA for the above-captioned PCT-application:

Enclosed please find new claims 1 to 30 amended under Article 34 PCT which should form the basis for the establishment of the IPER.

1. AMENDMENTS TO THE CLAIMS

1.1 New claim 1 corresponds to original claim 1, except for specifying the temperature (i.e. more than 41.2°C) and the time (i.e. at least 15 minutes) for inducing necrosis in tumor cells. This amendment is, inter alia, supported by original claims 3 and 7, the subject-matter of which has been incorporated into original claim 1.

VISITING ADDRESS:
Siebertstrasse 3
81675 Munich
Germany

POSTAL ADDRESS:
POB 86 07 67
81634 Munich
Germany

TEL.: +49-89-41 30 40
FAX: +49-89-41 30 41 11
+49-89-41 30 44 00 (Trademarks)
EMAIL: info@vossiusandpartner.com
HOMEPAGE: www.vossiusandpartner.com

1.2 New claims 2 to 30 correspond to original claims 2, 4 to 6 and 8 to 32 with adjusted back-references.

2. SUPPORT BY THE DESCRIPTION (ARTICLE 6 PCT AND RULE 6.3(A) PCT

2.1 In section 2.4 of the Written Opinion the Examiner states that original claims 1 and 2 do not contain the (essential) technical features which should define the invention so as to distinguish the invention from the prior art. Accordingly, the Examiner requests to introduce (a) feature(s) where upon not only novelty but also inventive step should be based on.

2.2 Original claim 1 has been amended insofar as the temperature and the time for inducing necrosis in tumor cells has been specified. Accordingly, new claim 1 meets the "support" requirement of Article 6 PCT and the "essential feature" requirement of Rule 6.3(a) PCT since the temperature for inducing necrosis in tumor cells has been specified to be more than 41.2°C and the time has been specified to be at least 15 minutes. These two parameters confer novelty and inventive step over the cited prior art documents (Gough (D1), Somersan (D2) and Kotera (D3)) as will be demonstrated in the following sections.

3. NOVELTY (ARTICLE 33(2) PCT)

3.1 In sections 2.3 and 2.5 of the Written Opinion the Examiner holds the view that original claims 1, 2, 18 to 20 and 26 to 29 lack novelty over Gough (D1), Somersan (D2) and Kotera (D3). The Examiner argues that D1 describes freeze/thawing and heat shock of tumor cells. D2 and D3 are said to also describe freeze/thawing of cells in order to obtain necrotic cells. Consequently, the Examiner concludes that original claims 1, 2, 18 to 20 and 26 to 29 lacked novelty.

3.2 The objection for lack of novelty raised against original claims 1, 2, 18 to 20 and 26 to 29 does not apply to new claims 1, 2, 16 to 18 and 24 to 27. This is because, none of D1 to D3 discloses the subject-matter of new claim 1 which relates to

> "A process for the production of an immunogenic compound comprising the steps of
> (a) inducing necrosis by a temperature of more than 41.2°C for at least 15 minutes; and
> (b) lysing said necrotic tumor cells so as to obtain a lysate." (emphasis added)

In particular, on page 7241, left column "Freeze-Thawing and Heat Shock of Tumor Cells", D1 only discloses that CMT93tk cells were transferred 3 times between liquid nitrogen and a 37°C water bath. Such cells were also lysed. In the same passage, D1 discloses that CMT 93tk cells were heat shocked at 42°C for 20 minutes and at 45°C for 1 hour, respectively. However, said heat shocked cells were not lysed, but used as living cells in co-cultivation with macrophages to show that said cells are able to stimulate the immune system; see page 7244, bottom of left column and Figure 6 C. Thus, D1 fails to disclose that necrosis is induced in tumor cells at a temperature of more than 41.2°C for at least 15 minutes and that so treated tumor cells are lysed.

D2 generates necrotic cells by repeated freeze/thawing steps (dry ice/ethanol and a 37°C water bath). Thus, D2 does not disclose that necrosis in tumor cells is induced at a temperature of more than 41.2°C for at least 15 minutes and wherein said necrotic tumor cells are lysed.

The same holds true for D3 which generates necrotic cell lysates by repeated freeze/thawing cycles without disclosing the temperature applied for thawing. Anyway, D3 fails to disclose that necrosis is induced by a temperature of more than 41.2°C.

In view of the above, we submit that new claim 1 is novel over D1 to D3. Since new claims 2, 16 to 18 and 24 to 27 (original claims 2, 18 to 20 and 26 to 29) depend directly or indirectly from new claim 1, they are novel over D1 to D3, too.

4. INVENTIVE STEP (ARTICLE 33(3) PCT)

4.1 In sections 2.3 and 2.5 of the Written Opinion the Examiner objects to original claims 1 to 32 as lacking inventive step over Gough (D1), Somersan (D2) and Kotera (D3).

4.2 However, in light of the amendment made to original claim 1, the new claims are not only novel, they also involve inventive step according to the technical problem-and-solution approach.

4.2.1 Gough (D1), could be seen as the closest prior art document. It discloses cell lysates and how macrophages orchestrate the immune response to tumor cell death. In that, D1 describes several experiments by, inter alia, using all lysates and heat shocked cells to show an immune response to tumor cells.

In D1 it was observed that macrophages phagocytosed both allegedly necrotic and apoptotic tumor cells, however, reacted differently with respect to said tumor cells. On one hand immunstimulatory cytokines, like TNFα or IL-1β were secreted if the macrophages phagocytosed necrotic cells, on the other hand immune-suppressive cytokines, like IL-10 were secreted if apoptotic cells were phagocytosed. Moreover, macrophages which were co-cultivated either with tumor cells incubated for 1 hour at 45°C or tumor cells incubated for 15 minutes at 45°C (both of which were not lysed) were tested for their cytokine secretion pattern. If challenged with tumor cells which were heat-induced for 1 hour, the macrophages reacted similarly like those challenged with chemically induced necrotic cells with respect to the secretion of cytokines. Whereas tumor cells which were heat-induced for 15

minutes resembled apoptotic cells in that they caused secretion of immune-suppressive cytokines after being phagocytosed by macrophages. To summarize, D1 discloses that lysates of tumor cells obtained by repeated freeze/thawing (liquid nitrogen/37° water bath) do not induce an immune response. Such lysates were only capable of inducing an immune response if exogenously added heat shock protein or endogenously expressed heat shock protein was present. An immune response was observed when tumor cells were heat shocked at 45°C for 60 minutes. However, so treated tumor cells were not lysed, but only co-cultured with macrophages.

Accordingly, D1 fails to disclose and/or teach that tumor cells are heat shocked at a temperature of more than 41.2°C for at least 15 minutes followed by a lysis which provides a necrotic cell lysate that is immunogenic although such cells do not necessarily contain a high amount of heat shock proteins which the art considered as being essential for inducing an immune response.

4.2.2 In light of the disclosure of the closest prior art document, the technical problem underlying the present application may be seen as the provision of means and methods for improved vaccination against cancers, tumorous diseases, infections and/or autoimmune diseases.

Said technical problem is solved by the provision of the means and methods for the production of an immunogenic compound comprising inducing necrosis in tumor cells by a temperature of more than 41.2°C for at least 15 minutes and the subsequent lysis of said necrotic tumor cells.

4.2.3 In light of the disclosure and teaching of the closest prior art document, it was not obvious to try to achieve said solution with a reasonable expectation of success.

As already pointed out in section 4.2.1, supra, D1 fails to show that cell lysates of freeze/thawed cells provide an immunogenic effect at all without exogenous or endogenous presence of heat shock proteins. Moreover, D1 does not show at all that cells in which necrosis has been induced at a temperature of more than 41.2°C for at least 15 minutes and which have

been lysed show any immunogenic effect. Furthermore, D1 fails to provide an incentive to lyse tumor cells after a heat shock at 45°C for 60 minutes. On the contrary, D1 uses living heat shocked cells which still secrete cytokines so as to observe an immunogenic effect of said heat shocked cells on macrophages.

The generation of lysates of heat shocked cells which, therefore, become necrotic distinguishes the present invention from the closest prior art document in which heat shocked cells were not lysed, but co-cultivated with machrophages to show that lethally heat shocked cells are able to stimulate the immune system; see Figure 6 on page 7244. Thus the difference between the present invention and D1 is, inter alia, that the present invention produces lysates of necrotic cells induced by heat shock for at least 15 minutes at a temperature of more than 41.2°C, whereas D1 uses living cells for this purpose.

Thus, since the skilled artisan when reading D1 is taught to use intact heat shocked cells to observe an immunogenic effect, he would not have taken into account using lysed, heat shocked cells. This is because, the skilled artisan is taught by D1 that the cells must still secrete cytokines in order to stimulate an immune response. This is why the authors of D1 used living heat-shocked cells to show the immunogenic activity of these cells. Freeze/thawed lysates also used in D1 were found to be only immune stimulating when Hsp was present (by supplementation or cell transfection). However, the present invention shows that, although heat shocked cells are lysed and, therefore, unable to produce and secrete immunogenic compounds, such lysed cells (i.e. lysates) are highly immunogenic.

Hence, it was neither obvious to try to provide the solution of the technical problem underlying the present invention nor was there a reasonable expectation of success. On the contrary, D1 rather teaches away from the present invention.

4.2.4 Likewise D1, neither D2 nor D3 renders the present invention obvious.
The freeze/thawing procedure mentioned in D2 or D3 results in cells that are quickly killed by submerging the cells in liquid nitrogen. Thawing the cells breaks the cellular membrane resulting in lysed cells. These cells are often called necrotic cells, however in fact these cells are just opened without induction of any necrotic process. In that, the present invention is distinguishable from D2 or D3 by the induction of necrosis using a temperature treatment above 41.2°C for at least 15 minutes. It is also important to mention that necrotic cells according to the present invention are fundamentally different from freeze/thawed cells that are also described as necrotic cells in the literature.

In particular, D2 teaches that tumor cell lysates which are enriched in Hsps induce maturation of human dendritic cells, while the present application teaches that necrotic tumor cell lysates harbouring less Hsps are unexpectedly high immunogenic both in vitro and in vivo.

A similar teaching is provided by D3 which uses untreated lysates of allegedly necrotic tumor cells to compare them to apoptotic tumor cell lysates. In fact, D3 teaches that untreated tumor cell lysates which harbour an amount of necrotic cell are comparable in their behaviour as a vaccine to apoptotic cell lysates. Yet, the present invention teaches that the more necrotic cells are contained in a cell lysate, the higher is the immunogenicity.
Moreover, D3 could not find a difference between allegedly necrotic cells, which were generated by freezing and thawing and apoptotic cells, which were generated by UV-B treatment when analysing the activation of dendritic cells. The same was observed in an animal model system when investigating the prophylactic and therapeutic efficacy of dendritic cells, which had taken up either the allegedly necrotic, or apoptotic cells.

It follows that neither D1 alone, nor a combination of D1 with D2 or D3 renders the subject-matter of the new claims obvious.

4.2.5 More importantly, the solution of the technical problem underlying the present invention bears unexpected and superior properties/effects over the prior art. The temperature and time applied to tumor cells for inducing necrosis serves for the surprising effect of the process of the present invention to induce necrosis in cells harbouring only a small amount of Hsps. Such cells show an unexpectedly high increase in immunogenicity in vitro and in vivo, although it was believed in the art that heat shocked cells must be living and that cells a high amount of heat shock proteins must be present to induce an immune response (cf. Tables 1 to 3 on pages 42 and 43 and Figures 3 to 5 as well as the "Results of in vitro/in vivo analysis" on pages 51 to 54 of the application as filed)

Thus, the temperature and time parameters recited in new claim 1 for inducing necrosis in cells independent of the presence of heat shock proteins renders the lysate obtained by the process of new claims 1 highly immunogenic which is surprising when looking at the teaching of the prior art. It is important to mention that the cell lysates produced in accordance with the teaching of the present invention are more immunogenic than untreated lysates although they do not contain Hsps. The immunogenic effect seems to be independent of the presence of Hsps. This is unexpected as Hsps are often made responsible, at least in part, for the immunogenic effect of a cellular vaccine (see D1 and D2). Furthermore, the advantage of lysing the heat-shocked cells becomes clear when the production of vaccines is considered. Lysed cells are much easier stored after production for later vaccinations and in case of tumor cell vaccination they can be vaccinated in patients without the need of an additional treatment to prevent tumor cell growth in the vaccinated patient.

5. MISCELLANEOUS

As requested in section 2.1 of the Written Opinion, we enclose Forms PCT/RO/134 for the deposited cell lines.

6. REQUESTS

In view of the amendments to the original claims and the above explanations, it is submitted that the defects set forth in the Written Opinion have been overcome. Accordingly, it is requested that a favorable IPER be issued.

If however, the Examiner wants to discuss our arguments in favor of novelty and inventive step over the phone, the undersigned is prepared to do so.

Dr. Hans-Rainer Jaenichen
European Patent Attorney

Enclosures: New claims on pages 55 to 58, in triplicate
Forms PCT/RO/134

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2605

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2606

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

CLAIMS

1. A process for the production of an immunogenic compound comprising the steps of
   (a) inducing necrosis by a temperature of more than 41.2°C for at least 15 minutes in tumor cells; and
   (b) lysing said necrotic tumor cells so as to obtain a lysate.

2. The process of claim 1, wherein necrosis is induced in tumor cells selected from the group consisting of tumor cell lines, cells derived from primary tumor material, cells derived from cell populations of primary tumor material and/or metastases including micrometastases.

3. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 42°C.

4. The process of any one of claims 1 to 3, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45°C to 55°C.

5. The process of any one of claims 1 to 4, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45.5°C to 47°C.

6. The process of any one of claims 1 to 5, wherein said induction of necrosis is performed in the range of 2 to 3 hours.

7. The process of any one of claims 1 to 6, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

8. The process of any one of claims 1 to 7, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

9. The process of any one of claims 1 to 8, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

10. The process of any one of claims 1 to 9, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

11. The process of any one of claims 1 to 9, wherein said tumor cells are allogeneic.

12. The process of any one of claims 1 to 9, wherein said tumor cells are syngenic.

13. The process of any one of claims 1 to 9, wherein said tumor cells are xenogenic.

14. The process of any one of claims 1 to 13, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

15. The process of any one of claims 1 to 14, wherein said tumor cells are NM-F9 cells (DSMZ deposit No DSM ACCC2606) or NM-D4 cells (DSMZ deposit No. DSM ACC2605).

16. A lysate obtainable by the process of any one of claims 1 to 15.

17. Dendritic cells loaded with the lysate of claim 16.

18. A composition comprising a lysate of claim 16 or dendritic cells of claim 17.

19. The composition of claim 18, which is a pharmaceutical composition.

20. The composition of claim 18, which is a vaccine composition.

21. The pharmaceutical composition of claim 20 or the vaccine composition of claim 20, which is optionally combined with an adjuvant.

22. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are immature.

23. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are mature.

24. A method for the production of a vaccine composition comprising the step of combining a cell lysate of claim 16 or the dendritic cells of claim 17 with an adjuvant.

25. A method for the production of a pharmaceutical composition comprising the step of combining a cell lysate of claim 16 with a pharmaceutically acceptable carrier.

26. A method for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases comprising administering a therapeutically or prophylactically effective amount of the lysate of claim 16 to an individual, or the pharmaceutical composition of claim 19, 21 or 22 or of the vaccine composition of any one of claims 20 to 22, or dendritc cells of claim 16.

27. Use of the lysate of claim 16 or of the dendritic cells of claim 17 for the preparation of a pharmaceutical or vaccine composition for the treatment or prevention of cancers, tumorous diseases, infections and/or autoimmune diseases.

28. The method of claim 26 or the use of claim 27, wherein said cancer or

tumorous disease is a cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer.

29. The method of claim 26 or the use of claim 27, wherein said infection is bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection.

30. The method of claim 26 or the use of claim 27, wherein said autoimmune disease is allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

CLAIMS

1. A process for the production of an immunogenic compound comprising the steps of
   (a) inducing necrosis by a temperature of more than 41.2°C for at least 15 minutes in tumor cells; and
   (b) lysing said necrotic tumor cells so as to obtain a lysate.

2. The process of claim 1, wherein necrosis is induced in tumor cells selected from the group consisting of tumor cell lines, cells derived from primary tumor material, cells derived from cell populations of primary tumor material and/or metastases including micrometastases.

3. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 42°C.

4. The process of any one of claims 1 to 3, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45°C to 55°C.

5. The process of any one of claims 1 to 4, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45.5°C to 47°C.

6. The process of any one of claims 1 to 5, wherein said induction of necrosis is performed in the range of 2 to 3 hours.

7. The process of any one of claims 1 to 6, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

8. The process of any one of claims 1 to 7, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

9. The process of any one of claims 1 to 8, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

10. The process of any one of claims 1 to 9, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

11. The process of any one of claims 1 to 9, wherein said tumor cells are allogeneic.

12. The process of any one of claims 1 to 9, wherein said tumor cells are syngenic.

13. The process of any one of claims 1 to 9, wherein said tumor cells are xenogenic.

14. The process of any one of claims 1 to 13, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

15. The process of any one of claims 1 to 14, wherein said tumor cells are NM-F9 cells (DSMZ deposit No DSM ACCC2606) or NM-D4 cells (DSMZ deposit No. DSM ACC2605).

16. A lysate obtainable by the process of any one of claims 1 to 15.

17. Dendritic cells loaded with the lysate of claim 16.

18. A composition comprising a lysate of claim 16 or dendritic cells of claim 17.

19. The composition of claim 18, which is a pharmaceutical composition.

20. The composition of claim 18, which is a vaccine composition.

21. The pharmaceutical composition of claim 20 or the vaccine composition of claim 20, which is optionally combined with an adjuvant.

22. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are immature.

23. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are mature.

24. A method for the production of a vaccine composition comprising the step of combining a cell lysate of claim 16 or the dendritic cells of claim 17 with an adjuvant.

25. A method for the production of a pharmaceutical composition comprising the step of combining a cell lysate of claim 16 with a pharmaceutically acceptable carrier.

26. A method for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases comprising administering a therapeutically or prophylactically effective amount of the lysate of claim 16 to an individual, or the pharmaceutical composition of claim 19, 21 or 22 or of the vaccine composition of any one of claims 20 to 22, or dendritc cells of claim 16.

27. Use of the lysate of claim 16 or of the dendritic cells of claim 17 for the preparation of a pharmaceutical or vaccine composition for the treatment or prevention of cancers, tumorous diseases, infections and/or autoimmune diseases.

28. The method of claim 26 or the use of claim 27, wherein said cancer or

tumorous disease is a cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer.

29. The method of claim 26 or the use of claim 27, wherein said infection is bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection.

30. The method of claim 26 or the use of claim 27, wherein said autoimmune disease is allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

CLAIMS

1. A process for the production of an immunogenic compound comprising the steps of
   (a) inducing necrosis by a temperature of more than 41.2°C for at least 15 minutes in tumor cells; and
   (b) lysing said necrotic tumor cells so as to obtain a lysate.

2. The process of claim 1, wherein necrosis is induced in tumor cells selected from the group consisting of tumor cell lines, cells derived from primary tumor material, cells derived from cell populations of primary tumor material and/or metastases including micrometastases.

3. The process of claim 1 or 2, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature of more than 42°C.

4. The process of any one of claims 1 to 3, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45°C to 55°C.

5. The process of any one of claims 1 to 4, wherein said induction of necrosis is achieved by incubating said tumor cells at a temperature in the range of 45.5°C to 47°C.

6. The process of any one of claims 1 to 5, wherein said induction of necrosis is performed in the range of 2 to 3 hours.

7. The process of any one of claims 1 to 6, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

8. The process of any one of claims 1 to 7, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

9. The process of any one of claims 1 to 8, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

10. The process of any one of claims 1 to 9, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

11. The process of any one of claims 1 to 9, wherein said tumor cells are allogeneic.

12. The process of any one of claims 1 to 9, wherein said tumor cells are syngenic.

13. The process of any one of claims 1 to 9, wherein said tumor cells are xenogenic.

14. The process of any one of claims 1 to 13, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

15. The process of any one of claims 1 to 14, wherein said tumor cells are NM-F9 cells (DSMZ deposit No DSM ACCC2606) or NM-D4 cells (DSMZ deposit No. DSM ACC2605).

16. A lysate obtainable by the process of any one of claims 1 to 15.

17. Dendritic cells loaded with the lysate of claim 16.

18. A composition comprising a lysate of claim 16 or dendritic cells of claim 17.

19. The composition of claim 18, which is a pharmaceutical composition.

20. The composition of claim 18, which is a vaccine composition.

21. The pharmaceutical composition of claim 20 or the vaccine composition of claim 20, which is optionally combined with an adjuvant.

22. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are immature.

23. The dendritic cells of claim 17 or the composition of claim 18, wherein said dendritic cells are mature.

24. A method for the production of a vaccine composition comprising the step of combining a cell lysate of claim 16 or the dendritic cells of claim 17 with an adjuvant.

25. A method for the production of a pharmaceutical composition comprising the step of combining a cell lysate of claim 16 with a pharmaceutically acceptable carrier.

26. A method for the treatment or prevention of cancer, tumorous diseases, infections and/or autoimmune diseases comprising administering a therapeutically or prophylactically effective amount of the lysate of claim 16 to an individual, or the pharmaceutical composition of claim 19, 21 or 22 or of the vaccine composition of any one of claims 20 to 22, or dendritc cells of claim 16.

27. Use of the lysate of claim 16 or of the dendritic cells of claim 17 for the preparation of a pharmaceutical or vaccine composition for the treatment or prevention of cancers, tumorous diseases, infections and/or autoimmune diseases.

28. The method of claim 26 or the use of claim 27, wherein said cancer or

tumorous disease is a cancer of the head and neck, lung, mediastinum, gastrointestinal tract, genitourinary system, gynaecological system, breast, endocrine system, skin, childhood, unknown primary site or metastatic cancer, a sarcoma of the soft tissue and bone, a mesothelioma, a melanoma, a neoplasm of the central nervous system, a lymphoma, a leukaemia, a paraneoplastic syndrome, a peritoneal carcinomastosis, a immunosuppression-related malignancy and/or metastatic cancer.

29. The method of claim 26 or the use of claim 27, wherein said infection is bacterial infection, viral infection, fungal infection, protozoal infection and/or helminthic infection.

30. The method of claim 26 or the use of claim 27, wherein said autoimmune disease is allergic encephalomyelitis, autoimmune haemolytic anemia, autoimmune thyroiditis, Hashimoto's disease, autoimmune male infertility, bullous pemphigoid, Celiac disease, Grave's disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, insulin-resistant diabetis mellitus, myasthenia gravis, pernicious anemia, pemphigus vulgaris, polyarteritis nodosa, primary biliary cirrhosis, Reiter's disease, rheumatic fever, sarcoidosis, Sjogren's disease, systemic lupus erythematosus, sympathetic ophthalmia, multiple sclerosis and/or viral myocarditis by Cocksakie B virus response.

PCT/EP2003/009140

REC'D 17 NOV 2003

WIPO PCT

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2605

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only

☐ This sheet was received with the international application

Authorized officer

For International Bureau use only

☒ This sheet was received by the International Bureau on:
17 NOVEMBER 2003

Authorized officer
EKO

Form PCT/RO/134 (July1998)

PCT/EP2003/009140

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

REC'D 17 NOV 2003
WIPO PCT

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NM-D4	DSM ACC2605

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-08-14 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address: Mascheroder Weg 1b
D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

U. Wirths

Date: 2003-10-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

PCT/EP2003/009140

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

REC'D 17 NOV 2003
WIPO PCT

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Nemod Biotherapeutics GmbH & Co. KG Address: Robert-Rössle-Str. 10 13125 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2605 Date of the deposit or the transfer[1]: 2003-08-14

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-08-18 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wecks Date: 2003-10-16

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 12/2001

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

REC'D 17 NOV 2003
WIPO PCT

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2606

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only

☐ This sheet was received with the international application

Authorized officer

For International Bureau use only

☒ This sheet was received by the International Bureau on:
17 NOVEMBER 2003

Authorized officer
EKO

PCT/EP2003/009140

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NM-F9	DSM ACC2606

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-08-14 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address: Maschcroder Weg 1b
D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

V. Wehs

Date: 2003-10-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

REC'D 17 NOV 2003
WIPO PCT

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Nemod Biotherapeutics GmbH & Co. KG Address: Robert-Rössle-Str. 10 13125 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2606 Date of the deposit or the transfer[1]: 2003-08-14

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-08-26 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2003-10-16

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

VOSSIUS & PARTNER

Vossius & Partner POB 86 07 67 81634 Munich Germany

Organisation Mondiale de la
Propriété Intellectuelle
Case postale 18

1211 Genève 20
SCHWEIZ

PCT-Patent Application PCT/EP03/09140
NEMOD Immuntherapie AG
Our Ref.: G2300 PCT S3

November 13, 2003
JAE/GW/HÄ

Pursuant to Rule 13*bis*PCT, in particular, Rule 13*bis*4(a)(i), we herewith furnish to the International Bureau according to Rule 13*bis*3 (ii) to (iv) the date of deposit of the biological material, the accession number given to the deposit by the respective institution and any further additional matter.

Accordingly, we enclose substitute pages 22, 23 and 56 which should replace previous pages 22, 23 and 56. Furthermore, we enclose the international forms for the receipt of the deposited biological material as well as the viability statement.

Finally, we enclose PCT-Form RO/134 for the deposited biological material.

Dr. Hans-Rainer Jaenichen
European Patent Attorney

Enclosure

cc.: European Patent Office, Munich

VISITING ADDRESS:
Siebertstrasse 3
81675 Munich
Germany

POSTAL ADDRESS:
POB 86 07 67
81634 Munich
Germany

TEL.: +49-89-41 30 40
FAX: +49-89-41 30 41 11
+49-89-41 30 44 00 (Trademarks)
EMAIL: info@vossiusandpartner.com
HOMEPAGE: www.vossiusandpartner.com

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Nemod Biotherapeutics GmbH & Co. KG Address: Robert-Rössle-Str. 10 13125 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2605 Date of the deposit or the transfer[1]: 2003-08-14

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-08-18 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2003-10-16

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NM-D4	DSM ACC2605

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-08-14 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH
Address:	Mascheroder Weg 1b D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

U. Wehs

Date: 2003-10-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG
Robert-Rössle-Str. 10
13125 Berlin

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NM-F9	DSM ACC2606

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-08-14 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address: Mascheroder Weg 1b
D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

V. Wehs

Date: 2003-10-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

INTERNATIONAL FORM

Nemod Biotherapeutics GmbH & Co. KG

Robert-Rössle-Str. 10

13125 Berlin

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Nemod Biotherapeutics GmbH & Co. KG Address: Robert-Rössle-Str. 10 13125 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2606 Date of the deposit or the transfer[1]: 2003-08-14

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-08-26 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss Date: 2003-10-16

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2605

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 22/23, line 27 to 29.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b
38124 Braunschweig
DE

Date of deposit	Accession Number
August 14, 2003	DSM ACC2606

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the EP patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

immunogenic compounds by inducing necrosis in the tumor cells and subsequently lysing the cells. The obtained tumor cell lysates can be used for the therapeutic or prophylactic treatment of cancer, tumorous diseases, infections and /or autoimmune diseases.

In this contex the term "immunogenic compound" means compounds having the ability to evoke immune reactions of the cells of the immune system like macrophages, dendritic cells, Langerhans' cells, B- (B1 and B2) and/or T-cells (cytotoxic T cells (Tc), T-helper cells (Th0, Th1 and Th2)), natural killer cells (NK cells), memory cells and the like Preferably, the term "immunogenic compound" means compounds having the ability to evoke humoral and/or cellular immune response, wherein at least one of the cells/group of cells of immune effector cells or cell products of said immune effector cells, for example one or more of the aforementioned cells are involved. The person skilled in the art is aware of various methods to determine whether an immune response is evoked. Examples for methods used for this purpose are shown in the examples and be transferred, where necessary by those skilled in the art, to determine the response in an individual or patient treated with the lysates according to the invention. Furthermore, other methods known to those skilled in the art can complement these techniques.

In general, an immunogenic compound leads to an immune response comprising humoral and/or cellular responses, normally comprising that genes or gene products that affect the level of immune responses are expressed/activated, e.g. those of the major histocompatibility class (MHC) I and II, those of antibody light and heavy chains, those of members of the immunglobulin superfamily, those of T-cell receptor/receptor compounds, those of cytokines or those of signal transduction cascades involved in transmitting immune responses.

In a preferred embodiment, the tumor cells used for the production of a cell lysate as described herein are NM-F9 (DSMZ deposit No. DSM ACC2606 or NM-D4 cells (DSMZ deposit No. DSM ACC2605)

The term "NM-F9" (also referred herein as "F9" or "TF-positive F9 cells") or "NM-D4" means cell lines or cells derived from the human myelogenous leukemia cell line K562 (ATCC: CCL-243). NM-F9 and NM-D4 were deposited with the Deutsche

Sammlung für Mikroorganismen und Zellkulturen GmbH ("DSMZ") on August 14, 2003. The DSMZ is located at the Mascheroder Weg 1b, D-38124 Braunschweig, Germany. The DSMZ deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

The present invention also relates to a lysate obtainable or obtained by the process according to the present invention and to dendritic cells loaded with such a lysate.

Moreover, the present invention also relates to a composition comprising a lysate or dendritic cells according to the present invention.

In a preferred embodiment said composition is a pharmaceutical composition. In accordance with the present invention the term "pharmaceutical composition " relates to compositions comprising the cell lysates described hereinabove which are obtained by the aforementioned processes and having the desired pharmacological activity. Such pharmaceutical compositions comprise a therapeutically effective amount of the cell lysates of the present invention, and a pharmaceutically acceptable carrier. The pharmaceutical composition may be administered with a physiologically acceptable carrier to a patient, as described herein. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also

9. The process of any one of claims 1 to 8, wherein more than 40% of said tumor cells are necrotic after induction of necrosis.

10. The process of any one of claims 1 to 9, wherein more than 70% of said tumor cells are necrotic after induction of necrosis.

11. The process of any one of claims 1 to 10, wherein said tumor cells are genetically engineered, mutated or infected by oncogenic viruses.

12. The process of any one of claims 1 to 11, wherein said tumor cells are autologous and from the same or from different tissues, organs or cell origin in a species.

13. The process of any one of claims 1 to 11, wherein said tumor cells are allogeneic.

14. The process of any one of claims 1 to 11, wherein said tumor cells are syngenic.

15. The process of any one of claims 1 to 11, wherein said tumor cells are xenogenic.

16. The process of any one of claims 1 to 15, wherein more than one type of tumor cell is used and wherein the tumor cells are from the same or different individuals, tissues, cell types or tumors.

17. The process of any one of claims 1 to 16, wherein said tumor cells are NM-F9 cells (DSMZ deposit No. DSM ACC2606) or NM-D4 cells (DSMZ deposit No. DSM ACC2605).

18. A lysate obtainable by the process of any one of claims 1 to 17.

19. Dendritic cells loaded with the lysate of claim 18.